Transplatin ineffectiveness against cancer from a molecular perspective: A single-molecule force-spectroscopy study

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By performing single-molecule force spectroscopy with optical tweezers, we have characterized the interaction between the platinum-based compound transplatin and the DNA molecule, establishing a critical comparison with its isomer cisplatin. While transplatin is ineffective against tumor cells, its isomer is one of the most used drugs in current chemotherapies, and a molecular study on this difference performed at the single-molecule level was lacking until the present work. Our experiments show that transplatin binds DNA under low chloride concentrations (a situation usually found inside many cells) with an equilibrium association binding constant about four orders of magnitude lower than cisplatin. In addition, we have found that, at saturation, transplatin binds preferentially forming interstrand cross links and monoadducts, a situation very different from cisplatin, which forms preferentially intrastrand cross links. Such differences explain the ineffectiveness of transplatin in killing tumor cells. From a physical point of view, the present study advances in using the mechanical properties of the DNA molecule as sensors to evaluate the therapeutic efficiency of drugs.

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I. INTRODUCTION

Platinum-based drugs are a class of compounds largely employed in cancer chemotherapies to treat various different types of tumors [1–7]. These drugs usually have the DNA molecule as their main target inside cells, forming various types of covalent adducts with the biopolymer, which inhibit basic cellular processes [8]. Cisplatin, for instance, was the first successfully tested platinum compound and is still used today in chemotherapies [9]. Curiously, its isomer (known as transplatin) is not effective against tumor cells. Although in the literature such difference is attributed to the types of covalent adducts formed between the drug and the DNA bases [10–13], it is still a subject of investigation [14].

Figure 1 shows a comparison between the structures of the two isomers. In aqueous solution, the chloride ions dissociate and the molecules incorporate water, reaching their cationic active state that interacts with DNA [8]. Both compounds can form single covalent adducts (monoadducts) or double covalent adducts (diadducts, also known as cross links) with two different bases, attaching these bases. It is reported that cisplatin forms preferentially 1,2-GG and 1,2-AG intrastrand cross links, i.e., diadducts between adjacent bases in the same strand [8,15]. Transplatin, on the other hand, is reported to form mainly monoadducts and 1,3-G*N*G-intrastrand cross links (N = any basis) due to the higher distance between the position of the chlorides in the molecule (see Fig. 1) [10,16].

Although there are some previous studies in the literature concerning the DNA interaction with transplatin [10–13,17], a force spectroscopy study of the DNA-transplatin complexes at the single molecule level is lacking. Such type of experiment is today recognized as the state-of-the-art for depicting DNA interactions with drugs and proteins [18,19], giving information about the binding modes and the physical chemistry of the interaction [19]. In particular, in the present case such a study can provide new insights on the differences between the mechanisms of action of the two isomers, giving clues about the ineffectiveness of transplatin in killing tumor cells.

Here we have performed single-molecule force spectroscopy assays with DNA-transplatin complexes in order to characterize the interaction in detail, comparing with cisplatin under identical experimental conditions. Our results show that, for low drug concentrations, there is a preference for the drug to bind forming monoadducts and intrastrand cross links. However, interstrand cross links become the most stable structures at higher drug concentrations. We performed the experiments using two buffers with very different chloride concentrations ([Cl]), showing that transplatin can exhibit an association equilibrium binding constant with DNA up to four orders of magnitude lower than cisplatin when [Cl] = 0. In addition, it is shown that transplatin binds without cooperativity in any situation, a result opposite to that previously reported for cisplatin [20,21]. Thus, the present study gives new insights on the differences between the mechanisms of action of the two isomers on the DNA molecule. In addition, the conclusions draw here can be helpful in the future development of new cisplatin and transplatin derivatives for chemotherapies. Finally, from a physical point of view, the present study advances in using the mechanical properties of the DNA molecule as sensors to evaluate the therapeutic efficiency of drugs.

II. MATERIALS AND METHODS

The assays were performed in two different phosphate buffered saline (PBS) solutions with very distinct ionic

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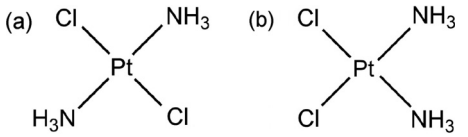

FIG. 1. Chemical structure of (a) transplatin and (b) cisplatin.

strengths, whose compositions are detailed in Table I. As important as the ionic strength itself for cisplatin and transplatin experiments is the concentration of chloride ions in solution ([Cl]), since this quantity determines the concentration of cisplatin and transplatin that are in the active state in solution: low values of [Cl] in solution favors the dissociation of the chloride ions from the drug molecules, thus increasing the concentration of drug found in the active state (which binds to DNA [8]). The effects of the chloride concentration and ionic strength of the buffer are in fact different. The presence of Cl^- ions in the buffer is related to the aquation reaction of transplatin (and cisplatin). Low chloride concentrations in the buffer promote the aquation of the drugs, allowing them to reach their active bivalent (+2) state, which binds to DNA, as mentioned before. Thus, increasing the chloride concentration in the buffer will result in less aquated transplatin molecules, which is equivalent to a reduction on the effective drug concentration in the sample, since only the drug molecules in the active state interacts with DNA. The direct interaction of the active drug with DNA, on the other hand, surely depends on the ionic strength, because more counterions screens the electrostatic interaction between the cationic active drugs and the negative phosphate backbone of the double helix.

The optical tweezers used here consist of a 1064 nm solid-state laser (Altechna Corp.) mounted in a Nikon Ti-U inverted microscope with a 100× N.A. 1.4 objective. Laser power was adjusted to 30 mW at the objective entrance, which results in a small trap stiffness (∼3pN/μm) that is adequate to stretch DNA with low forces (<3 pN), i.e., within the entropic regime.

The samples are prepared tethering biotin-labeled λ-DNA molecules (48502 base pairs, ∼16.5 μm contour length) by the ends between a streptavidin-coated cover lip and a streptavidin-coated polystyrene bead with 3 μm diameter. The tweezers is then used to trap this bead and pull DNA with controlled velocity using a piezoelectric device (Newport), allowing one to obtain the force-extension curve (FEC) of the biopolymer. The FECs were fitted to the Marko-Siggia wormlike chain (WLC) equation [22], allowing one to extract the persistence and contour lengths of the DNA molecule and of its complexes formed with transplatin, at various drug concentrations. The complete details of these procedures can be found in previously published works [23]. In Fig. 2 we show some FECs obtained under our experimental conditions along with the WLC fittings (solid lines), for the two buffers used. Observe that the WLC model fits very well to our experimental data, allowing the determination of the mechanical properties (persistence and contour lengths) with accuracy.

TABLE I. Composition of the different PBS buffers used.

Buffer	NaCl	Na_2HPO_4	NaH_2PO_4
PBS [Cl] = 140 mM	140 mM	4.375 mM	1.25 mM
PBS [Cl] = 0 mM	0	0.4375 mM	0.125 mM

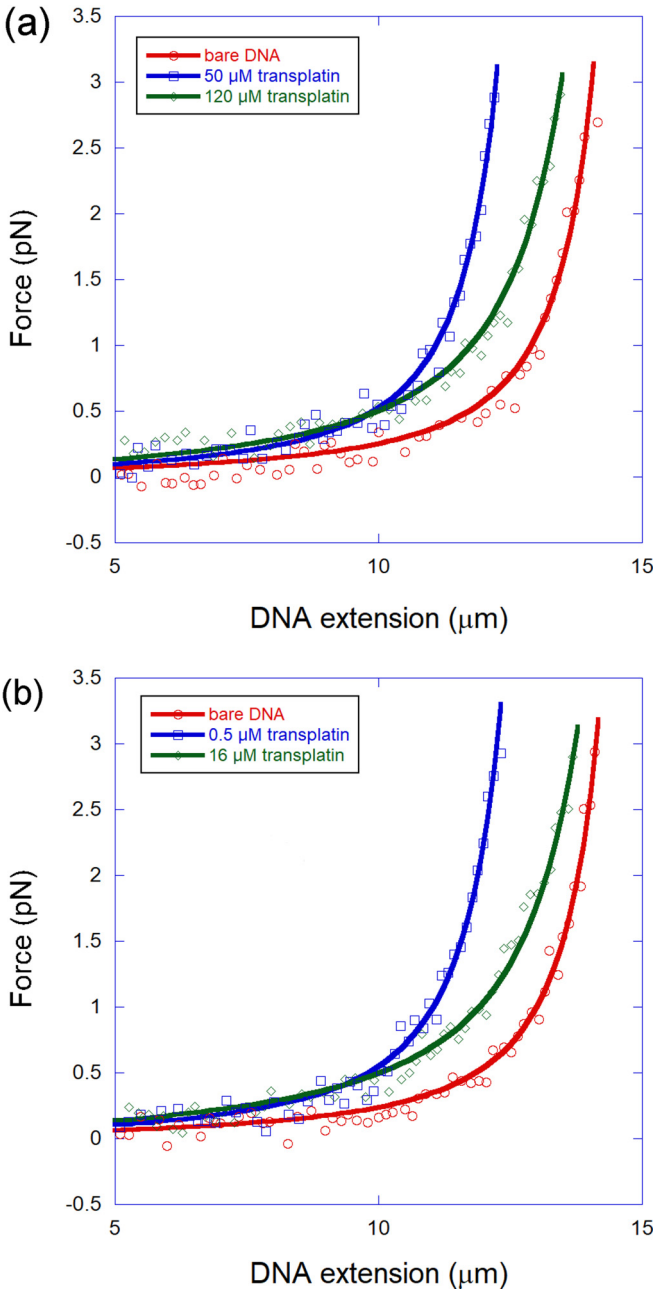

FIG. 2. (a) Exemplifying force-extension curves (FECs) along with the WLC fittings (solid lines) for the PBS with [Cl] = 140 mM and (b) for the PBS without [Cl]. Observe that the WLC model fits very well to our experimental data, allowing the determination of the mechanical properties (persistence and contour lengths) with accuracy.

All samples and stocks containing transplatin were prepared immediately before use to avoid degradation. The drug was left to equilibrate with DNA for ∼30 min for each concentration. Such time was sufficient for drug equilibration under our experimental conditions. In fact, we have performed

some tests repeating the experiments with longer incubation times, obtaining similar results. All experiments were performed with the same DNA molecule, gradually changing the drug concentration during the experiments. In other words, the working DNA is maintained tethered in the tweezers during the assay while changing the drug concentration, which guarantees that the changes on the mechanical properties are evaluated over the same molecule. Thus, the error bars reported for the mechanical parameters are the standard error calculated from a series of repeated measurements over the same DNA molecule, although we have also repeated the complete experiment (using all the different drug concentrations) with at least ten different DNA molecules in order to evaluate the reproducibility of the results.

III. RESULTS AND DISCUSSION

A. Mechanical behavior of the DNA-transplatin complexes determined from single-molecule force spectroscopy.

Single-molecule force spectroscopy experiments with optical tweezers were performed here with the complexes formed between DNA and transplatin. The DNA-transplatin complexes were stretched with low forces (<3 pN) and the mechanical properties (contour and persistence lengths) were determined by WLC fitting, as mentioned before. Figure 3 shows the measured contour length of the DNA complexes formed with transplatin in the two buffers used here. Figure 4 shows the corresponding persistence lengths of these complexes.

Figure 3(a) specifically shows the measured contour length of the complexes as a function of the drug concentration for [Cl] = 140 mM. Observe that this mechanical property exhibits an unusual nonmonotonic behavior, decreasing for low concentrations (<50 μM) but increasing again for higher concentrations (>50 μM). Such behavior suggests that the binding mode changes with the transplatin concentration under our experimental conditions. Probably, at low drug concentrations (<50 μM) transplatin binds preferentially forming intrastrand cross links, which can approximate different DNA segments and thus decrease the apparent contour length measured at the low-force entropic regime [21]. Certainly there are also many monoadducts formed along the double helix at this situation, but such structures do not change the contour length since they do not modify the average distance between DNA base pairs. On the other hand, for higher drug concentrations (>50 μM), the increase measured for the contour length indicates that the intrastrand cross links are not anymore the most stable binding mode at these concentrations, and may be rearranged into more stable interstrand cross links [17]. Interstrand cross links in general do not approximate distant DNA base pairs, because the distance between the drug binding sites (positions of the Cl) is not too much larger than the diameter of the double helix. Thus, at this configuration the drug tends to bind to base pairs in opposite strands that are spatially close. Therefore, the contour length tends to return to its original value as intrastrand cross links are substituted by the more stable interstrand ones. Such proposal corroborates with previous works from other groups obtained using different experimental techniques [10,14]. In particular, the results of Kishimoto *et al.* obtained using fluorescence microscopy show that the

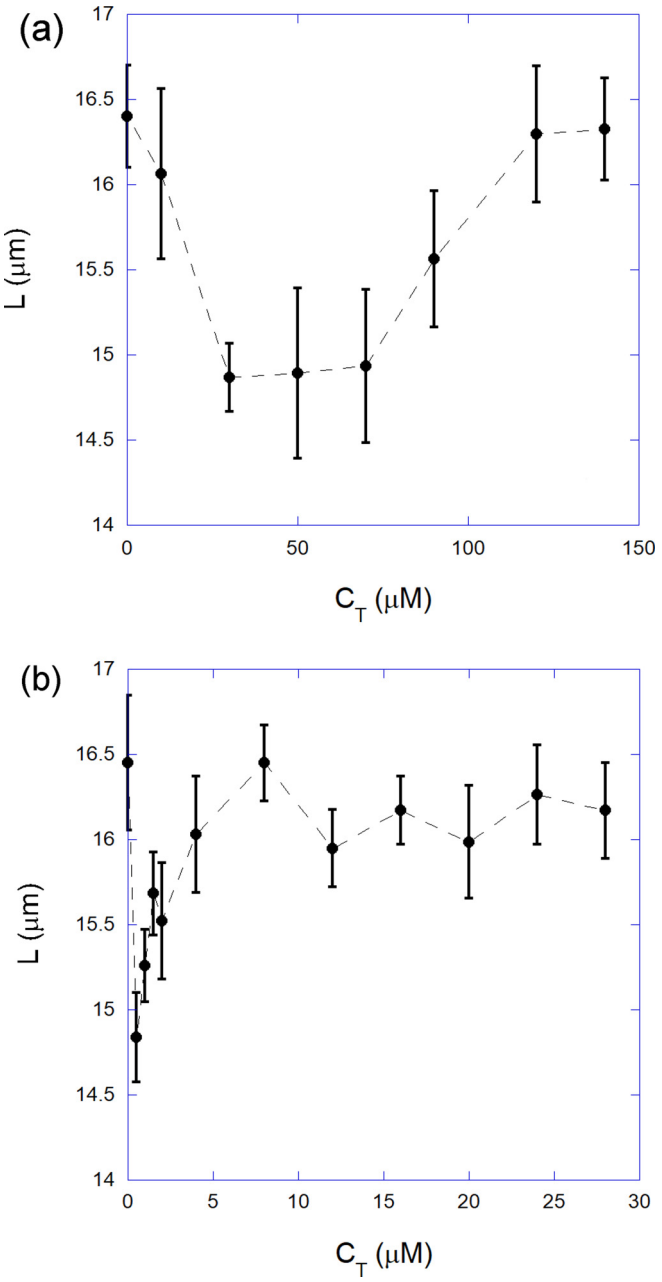

FIG. 3. (a) Contour length of the DNA-transplatin complexes measured as a function of the drug concentration in the sample for the PBS with [Cl] = 140 mM and (b) for the PBS without [Cl].

DNA size (measured as the average long axis of the molecule and as the hydrodynamic radius) presents a nonmonotonic behavior as a function of the quantity of bound transplatin [14], in agreement with the present study.

Figure 3(b) shows the contour length of the DNA-transplatin complexes measured as a function of the drug concentration in the buffer without [Cl]. The qualitative behavior is similar to that found for the previous case ([Cl] = 140 mM). Nevertheless, the contour length saturates at a much lower drug concentration, indicating that transplatin is more reactive at low ionic strengths, as will be evident later.

Figure 4(a) shows the persistence length of the DNA-transplatin complexes measured as a function of the drug

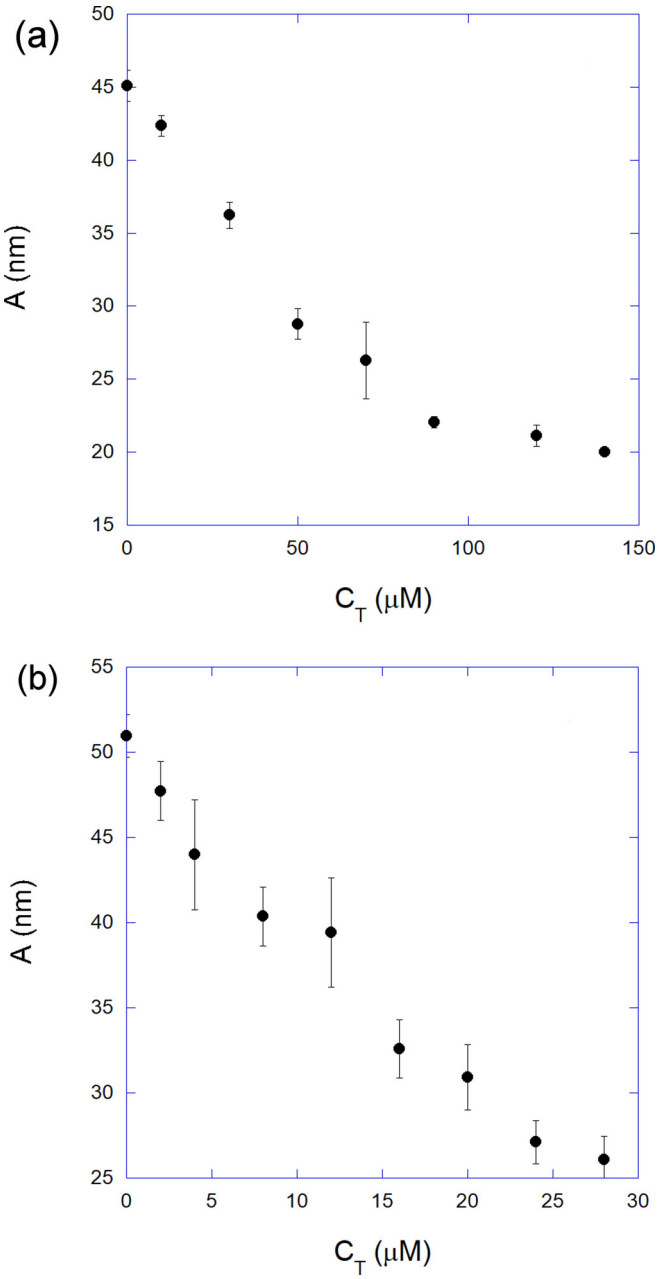

FIG. 4. (a) Persistence length of the DNA-transplatin complexes measured as a function of the drug concentration in the sample for the PBS with [Cl] = 140 mM and (b) for the PBS without [Cl].

concentration for [Cl] = 140 mM. This mechanical parameter decreases monotonically as more drug binds to the double helix, indicating that any type of covalent adduct formed decreases the bending stiffness of DNA, a result compatible to those previously found for cisplatin [20] and other platinum-based compounds derived from cisplatin [24,25].

Figure 4(b) shows the persistence length of the DNA-transplatin complexes measured as a function of the drug concentration in the buffer without [Cl]. The qualitative behavior is very similar to that found for the previous case ([Cl] = 140 mM), and the concentration range in which the persistence length decreases again suggests that transplatin is more reactive at low ionic strengths.

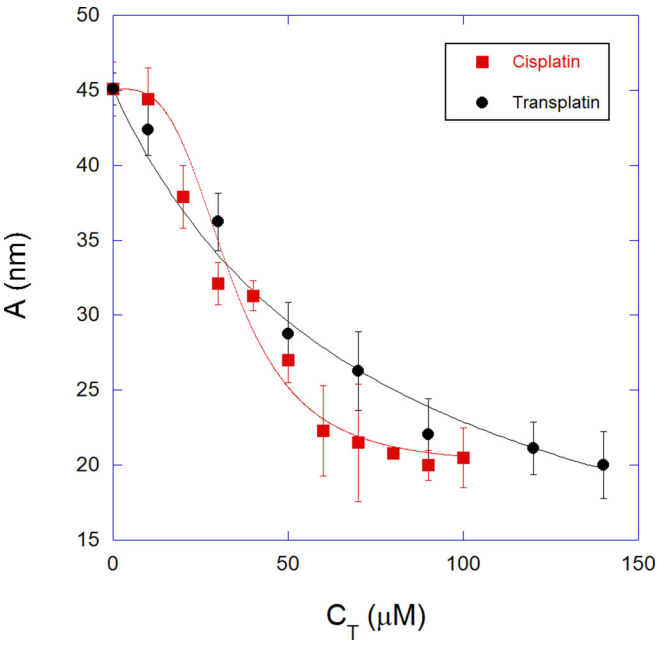

FIG. 5. Persistence length and model fittings (solid lines) for the two isomer drugs, obtained for [Cl] = 140 mM. The binding parameters that characterize the interactions are summarized in Table II.

In the next section we use our quenched-disorder statistical model for ligand binding [19,26] in order to determine the binding parameters of the DNA-transplatin interaction in the two different buffers. A critical comparison with cisplatin is also provided in order to understand the difference between the two isomers in their interaction with DNA, which lead to the well-known difference in the efficacy for killing cancer cells. Briefly, our quenched-disorder model provides an equation for the effective persistence length of the resulting DNA-ligand complex as a function of the ligand concentration in the sample, such that it can fit the experimental data of Fig. 4 [19]. From this fitting, one can determine the physicochemical (binding) parameters of the interaction. Thus, the methodology allows one to determine the physical chemistry of the interaction from single-molecule force-spectroscopy assays.

B. Determining the binding parameters of the interaction: A direct comparison with the isomer cisplatin.

In Figs. 5 and 6 we show the persistence length data of the DNA-transplatin complexes fitted with our quenched-disorder statistical model of ligand binding using a Hill-type binding isotherm [19,26], for the two buffers used here. Such model

TABLE II. Binding parameters determined from the fittings for transplatin and cisplatin.

Drug	Buffer ([Cl])	K (M^{-1})	n
Transplatin	140 mM	$(1.8 \pm 0.2) \times 10^3$	1.0 ± 0.1
Cisplatin	140 mM	$(2.4 \pm 0.4) \times 10^4$	3.4 ± 0.4
Transplatin	0	$(4.8 \pm 0.4) \times 10^3$	1.0 ± 0.1
Cisplatin	0	$(1.5 \pm 0.4) \times 10^7$	1.0 ± 0.2

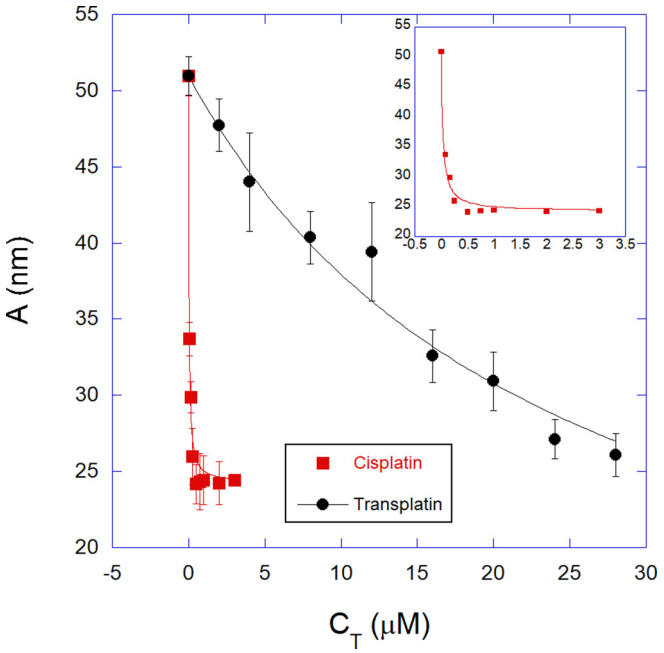

FIG. 6. Persistence length and model fittings (solid lines) for the two isomer drugs, obtained in the buffer without [Cl]. The binding parameters that characterize the interactions are summarized in Table II. Inset: rescaled cisplatin data to improve visualization.

was exhaustively used in previous works for other DNA-drug systems and allows one to determine the binding parameters of the interaction from the persistence length data as a function of the drug concentration in the sample. A complete review of this methodology was recently published [19].

The equivalent results for cisplatin previously published [20] are also shown in the figures for comparison purposes. The values of the binding parameters obtained from the fittings are summarized in Table II. From these fittings we can draw the following conclusions:

(i) At high [Cl] (140 mM) the concentration ranges in which the two drugs interact with DNA (changing its mechanical properties) are similar, as can be seen in Fig. 5. In fact, the fittings return the equilibrium binding association constants, which are $K_{\text{trans}} = (1.8 \pm 0.2) \times 10^3\ M^{-1}$ and $K_{\text{cisp}} = (2.4 \pm 0.4) \times 10^4\ M^{-1}$. These results show that cisplatin binds to DNA with an equilibrium association constant about one order of magnitude higher than transplatin at this situation (high ionic strength, [Cl] = 140 mM).

(ii) Also in Fig. 5, it can be seen that while the persistence length exhibits a sigmoidal decay for cisplatin, such behavior does not occur for transplatin. From a biochemical point of view, this fact means that cisplatin binds cooperatively to DNA, while transplatin binds without cooperativity under such conditions. This is reflected by the Hill coefficient returned by the fittings, which were $n_{\text{trans}} = 1.0 \pm 0.1$, confirming a noncooperative interaction, and $n_{\text{cisp}} = 3.4 \pm 0.4$, indicating positive cooperativity.

(iii) In the buffer without [Cl] (Fig. 6), observe that the relevant concentration ranges of the two drugs are very different, and cisplatin saturates at a much lower concentration than transplatin, reflecting a much higher association constant. The fittings in fact return in this case $K_{\text{trans}} = (4.8 \pm 0.4) \times 10^3\ M^{-1}$ and $K_{\text{cisp}} = (1.5 \pm 0.4) \times 10^7\ M^{-1}$. Thus, at this situation cisplatin binds with an association constant about four orders of magnitude higher than transplatin. In addition, note that both drugs induce a nonsigmoidal decay on the persistence length, indicating that the cooperativity exhibited by cisplatin in the previous situation was lost, as previously reported [20].

Inside many cells, it is known that the concentration of chloride ions is relatively small (a few mM) [8]. We have shown here that, under this situation, cisplatin is much more reactive than transplatin, exhibiting an equilibrium association constant about four orders of magnitude higher. Such high difference on the binding constant can explain in part why cisplatin is much more effective to treat tumors. In fact, most anticancer drugs present equilibrium constants (K) with DNA between $10^4\ M^{-1}$ to $10^7\ M^{-1}$ [18,19]. While cisplatin is among the drugs with highest values of K for low [Cl], transplatin presents a value of K below the typical range of currently used drugs. In addition, note that transplatin is practically insensitive to the changes of ionic strength and chloride concentration, presenting equilibrium association constants with the same order of magnitude in the two buffers.

The other aspect reported in the literature as responsible for explaining the much lower effectiveness of transplatin in killing cancer cells is the difference in the types of adducts formed with DNA [10,11,17]. Here we have concluded from the data of Fig. 3 that, at saturation, transplatin may form mainly interstrand cross links and monoadducts. Cisplatin, on the other hand, form mainly intrastrand cross links. Therefore, such difference can also be a relevant factor for explaining the action of the two compounds inside cells. An intriguing question is: Would transplatin be effective in killing tumor cells at low concentrations, since the data of Fig. 3 suggest that intrastrand cross links are favored at low drug concentrations? Although the answer obviously depends on further research, there are studies that suggest that transplatin may be efficient at low concentrations, especially when combined with cisplatin [12]. The main problem is the fact that, even if transplatin is effective *in vitro* under these low concentrations for interacting with a very exposed DNA molecule, there is no guarantee that such low concentrations will work *in vivo*. In addition, there are studies reporting that transplatin is more susceptible to react with glutathione, metallothionein and other sulfur-containing molecules inside cells, reducing the amount of drug available to bind DNA [27,28].

IV. CONCLUSION

We have characterized the interaction between the platinum compound transplatin and the DNA molecule at the single-molecule level by performing force spectroscopy with optical tweezers. A critical comparison with the isomer cisplatin, largely employed in chemotherapies, was also done. We have found that transplatin binds DNA under low chloride concentrations (the situation usually found inside many cells) with an equilibrium binding constant about four orders of magnitude smaller than cisplatin. This result explains, at least

partially, why transplatin is ineffective in killing tumor cells, although the ionic strength inside cells is not as low as the one used here in the buffer without [Cl]. What is really important here is the comparison between transplatin and cisplatin under identical experimental conditions, which was performed in the present study for two buffers with very different characteristics. Note that transplatin presents a smaller equilibrium binding constant than cisplatin in any situation. Even for the higher ionic strength buffer, the difference is about one order of magnitude, which is high enough, and tends to increase as the chloride concentration is lowered in the environment. In addition, it should be noted that transplatin is practically insensitive to the changes of ionic strength and chloride concentration in the buffer, a result very different to that found for cisplatin.

Finally, we have also found that, at saturation, transplatin tends to bind forming interstrand cross links and monoadducts, a situation very different from cisplatin, which forms preferentially intrastrand cross links. This difference may be also related to the effectiveness of the two compounds. At low drug concentrations, however, transplatin binds preferentially forming intrastrand cross links, which suggests an explanation for the surprising efficacy of the compound at low concentrations reported in the literature [12].

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