Transition on the entropic elasticity of DNA induced by intercalating molecules

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We use optical tweezers to perform stretching experiments on DNA molecules when interacting with the drugs daunomycin and ethidium bromide, which intercalate the DNA molecule. Our results show that the persistence length of the DNA-drug complexes increases strongly as the drug concentration increases up to some critical value. Above this critical value, the persistence length decreases abruptly and remains approximately constant for larger drug concentrations, at least in the concentration range used in our experiments. Measured intercalators critical concentrations for the persistence length transition coincide with the reported values for the helix-coil transition of DNA-drug complexes obtained from sedimentation experiments. The contour length of the molecules increases monotonically and saturates as the drug concentration increases. The neighbor exclusion model fits to our results for the total drug concentration as a function of the relative increase of the contour length. © *2007 American Institute of Physics*. [DOI: 10.1063/1.2768945]

I. INTRODUCTION

DNA-drug interactions have been much studied for the past years. An important motivation for these studies is the fact that many of the studied drugs are used for treatment of human diseases, particularly, in cancer chemotherapy.

Single molecule stretching experiments using optical tweezers have yielded a great amount of information about DNA-protein and DNA-drug interactions.[1–6]

Recently, we studied the interaction between psoralen and DNA when illuminated with ultraviolet light A (UVA). Psoralen is a drug used to treat some skin diseases, such as psoriasis and vitiligo. This drug intercalates the DNA molecule and can form covalent linkages with the thymines if the complex is illuminated with ultraviolet light, modifying drastically its elasticity and impeding the DNA replication and transcription. The persistence length of the DNA-psoralen complexes formed after UVA illumination was measured in Ref. 7.

Daunomycin and ethidium bromide (EtBr) are other examples of drugs which intercalate the DNA molecule and can modify its elasticity, depending on the drug concentration. Both drugs unwind the DNA double helix when intercalating.[8] Daunomycin is an anthracycline antibiotic used in the treatment of various cancers. It inhibits DNA replication and transcription when intercalating, impeding cell duplication.[9] Ethidium bromide (EtBr) is commonly used as a nonradioactive marker for identifying and visualizing nucleic acid bands in electrophoresis and in other methods of nucleic acid separation.

Several works have reported different results for the effects of these drugs on the entropic elasticity of DNA molecules. In those works, the measured parameter used to study elasticity modifications is the persistence length of the DNA-drug complex. Smith *et al.*[2] reported that ethidium bromide does not modify the elasticity of the DNA molecule; it only increases its contour length by ~40%. Tessmer *et al.*[10] reported that ethidium bromide causes a large increase in the contour length and a decrease in the persistence length of the complex for 1 μM of the drug, and at lower concentrations, an increase in both persistence and contour lengths. Sischka *et al.*[11] reported the value of 28.1 nm for the persistence length of DNA-daunomycin complexes and 20.7 nm for DNA-EtBr complexes, smaller than the bare DNA persistence length of about 50 nm. The authors in this work have used a concentration of 1 μM for both drugs, and a DNA concentration of 15 pM. Recently, Vladescu *et al.*[12] reported the value of 5.7 nm for the persistence length of highly saturated DNA-EtBr complexes. In the present work, in order to clearly establish the effect of these intercalating drugs on the persistence length of the DNA complexes, we performed stretching experiments at various drug concentrations, from zero up to saturation of the complexes. We show that the values obtained for the persistence length depend strongly on the concentration ratio between drug and DNA base pairs. Our results show that the persistence length of the complexes increases as we increase the drug concentration until certain critical concentration is reached. Above this critical concentration the persistence length decreases abruptly and remains practically constant for larger drug concentrations.

II. EXPERIMENTAL PROCEDURE

To measure the persistence and contour length of DNA molecules and DNA-drug complexes, we use optical tweezers[1–6] and intensity autocorrelation spectroscopy.[13]

The samples consist of λ-DNA molecules in a phosphate buffered saline *p*H 7.4 with [NaCl]=140 mM solution. We attach one end of the molecule to a microscope coverslip, and the other end is attached to a polystyrene bead. To do this, we use the procedure described in Ref. 14. The DNA concentration used in all experiments was C_{DNA}

=6.81 μg/ml, which corresponds to a base pairs concentration of C_{bp}=11 μM. In the procedure used to prepare the samples, only 5%–10% of DNA attach simultaneously both to the coverslip and to one bead (this is the condition that we needed to perform the measurements). The sample is always prepared with bare DNAs and incubated for ~12 h. The drug is added to the sample after this time and we waited for at least 1 h to start the measurements. The concentration of free DNA in the solution (which is not attached at least to one bead or to the coverslip) is very low, since the incubation time is very long.

Our optical tweezers is mounted in a Nikon TE300 microscope with an infinite corrected objective (100×, numerical aperture=1.4). The trapping laser is an infrared (IR) laser with λ=832 nm (SDL, 5422-H1). The optical tweezers is used to trap a polystyrene bead of 2.8 μm diameter attached to the end of the DNA molecule, so we can manipulate and stretch the DNA molecule.

In addition, we use a He–Ne laser (λ=632.8 nm) as the scattering probe. The backscattered light by the polystyrene bead is collected by a photodetector, which delivers pulses to a digital correlator. We, then, obtain the autocorrelation function of the backscattered light, which allows us to determine the stiffness of the optical trap, due to the Brownian motion of the trapped bead.

The next step in the experimental procedure is to obtain the force versus extension curves for the DNA molecules and DNA-drug complexes. To do this, we use the optical tweezers to trap the bead with the DNA while pulling the microscope slide, stretching the DNA. The backscattered light is collected over time while stretching the DNA. From the backscattered light intensity, one obtains the displacement of the trapped bead in relation to its equilibrium position, and by multiplying it by the tweezers' stiffness, the force exerted by the DNA molecule while it is stretched is obtained. The DNA extension is obtained knowing the pulling velocity of the coverslip (58 nm/s). This velocity is low enough to guarantee that the DNA passes through equilibrium configurations and low enough such that the Stokes force on the bead is negligible. Therefore, we transform time from the data into absolute extension along the x axis (parallel to the coverslip), which we call x_{DNA}. This procedure is done for all measurements, even on repeated measurements with the same molecule. The bead center is maintained at a fixed distance (h =3.5 μm) from the coverslip during all measurements. The end-to-end distance z of the DNA molecule is related to x_{DNA} by $z=\sqrt{x_{DNA}^2+h^2}$.

Finally, with the force versus extension curves, we use the approximate expression derived by Marko and Siggia[15] [Eq. (1)] to obtain the persistence and contour length of the DNA molecules and DNA-drug complexes,

$$F_x = \frac{k_B T}{A}\left[\frac{\sqrt{x_{DNA}^2+h^2}}{L} + \frac{1}{4[1-(\sqrt{x_{DNA}^2+h^2})/L]} - \frac{1}{4}\right] \times \frac{x_{DNA}}{\sqrt{x_{DNA}^2+h^2}}, \quad (1)$$

where F_x is the x component of the force, k_B is the Boltzmann constant, T is the absolute temperature, A is the DNA persistence length, L is the DNA contour length, x_{DNA} is the projection of DNA molecule end-to-end distance along the x axis (parallel to the coverslip), and h is the height of the bead relative to the coverslip (distance between the center of the bead and the coverslip). The height is fixed in all experiments at h=3.5 μm, and we measure x_{DNA}.

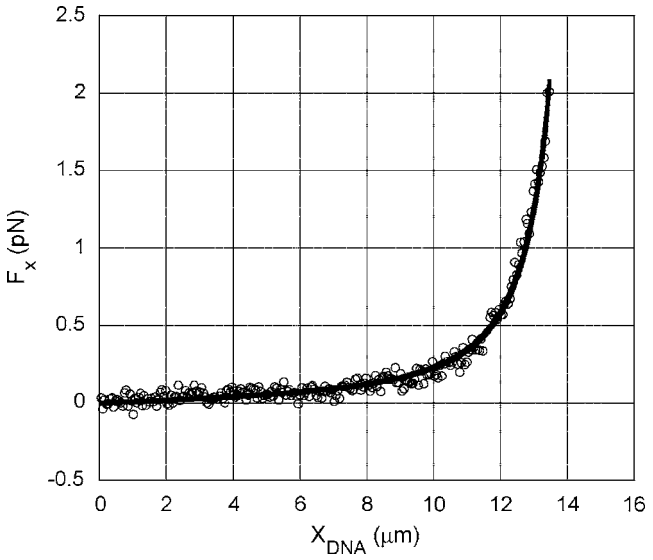

FIG. 1. Force as a function of extension for a drug-free DNA molecule. By fitting this curve to Eq. (1), we determine the persistence length A =49.9 nm and the contour length L=15.5 μm for this particular λ-DNA.

The details about our experimental setup and experimental procedure can be found in Refs. 7 and 13.

Figure 1 is a typical force versus extension curve obtained with this procedure, for a bare DNA molecule. By fitting this curve with Eq. (1), we extract the persistence length and the contour length for the λ-DNA. For the particular DNA molecule shown in Fig. 1, we find A=49.9 nm and L=15.5 μm.

Observe that we limit the range of forces used to ≲2 pN. We have limited our measurements to low forces because higher forces may change the chemical equilibrium of the DNA-drug complex. Also, lower forces avoids enthalpic effects which are not taken into account in Eq. (1). To show that the forces used in our experiments are sufficient for determining the persistence length, we repeat several times the measurement of the persistence length for a single bare DNA molecule over time. The values obtained in several different runs and different DNAs are A=50±3 nm and L =16.5±1 μm. These values correspond to the well-known values reported in the literature.[3,16,17] This result indicates the accuracy of our method, and shows that the experimental apparatus do not exhibit significant drifts.

To determine the error bars of both persistence and contour lengths, we use the following procedure. We first perform five measurements (different force×extension curves) with the same DNA molecule (or DNA-drug complex). The data of these curves are summed and averaged to a single curve for this complex. The fit to Eq. (1) then gives the values of both A and L for the complex, as in Fig. 1 for a bare DNA. We then repeat the same procedure for five dif-

ferent complexes, obtaining the values for both A and L for each complex. The presented values of A and L are averages over the different values obtained for each complex. We then determine the error bars by calculating the standard deviation from the mean value. The variability of L for different complexes is relatively large, but the resulting A varies very little, indicating that L and A are little correlated for $L \gg A$. This fact occurs because the contour length of bare λ-DNA molecules is not always 16.5 μm. Performing several measurements, one can find different results around this value. Such distribution of contour length values was also observed by Mihailovic *et al.*[18]

III. RESULTS

In this section we show the results obtained for the two drugs used: daunomycin and ethidium bromide.

A. Daunomycin

We have performed experiments with DNA-daunomycin complexes for several drug concentrations. In Fig. 2(a) we show the persistence length (A) of the complexes as a function of total daunomycin concentration (C_D) for fixed DNA base pair concentration of $C_{bp}=11$ μM. We denote by C_D the total daunomycin concentration used to prepare the sample, which is the sum of both the bounded to DNA and the free drug concentration in the solution.

The point which the drug concentration is zero corresponds to the drug-free DNA situation with $A=50\pm3$ nm.

The behavior of the persistence length A as a function of daunomycin concentration C_D can be described as follows: it initially increases with C_D until it reaches a maximum value (~280 nm) at the critical concentration $C_D^{\text{critical}}=18.3$ μM. Then, it decays abruptly to around 75 nm and remains approximately constant at this value for the concentration range used.

The contour length, otherwise, increases monotonically from 16.5 ± 1 μm up to the saturation value of 21 ± 1.8 μm. In Fig. 2(b) we plot the corresponding values of the contour length of the same DNA-drug complexes of Fig. 2(a) as a function of the total drug concentration C_D. As we have pointed out in Sec. II, the error bars of the contour length are relatively large as a consequence of the contour length distribution around 16.5 μm.[18]

In addition, we can estimate the exclusion parameter n (number of total base pairs divided by the number of total intercalated drug molecules) from our experimental data. The average value of the contour length for DNA-daunomycin complexes obtained when using a saturated concentration of the drug increases about 27% relative to drug-free DNA contour length (16.5 μm). This means that when all possible drug molecules are intercalated, the DNA increases its contour length by 4.5 μm. Knowing that each intercalated daunomycin molecule increases the contour length of the complex by 0.31 nm,[8] we determine the total number of intercalated daunomycin molecules, which is around 14 500. Finally, the exclusion parameter can be obtained by dividing the number of base pairs of the λ-DNA (48 500) by the number of total intercalated drug molecules (14 500). We obtain $n=3.3$, which is in good agreement with the value of 3.5 reported in Ref. 9.

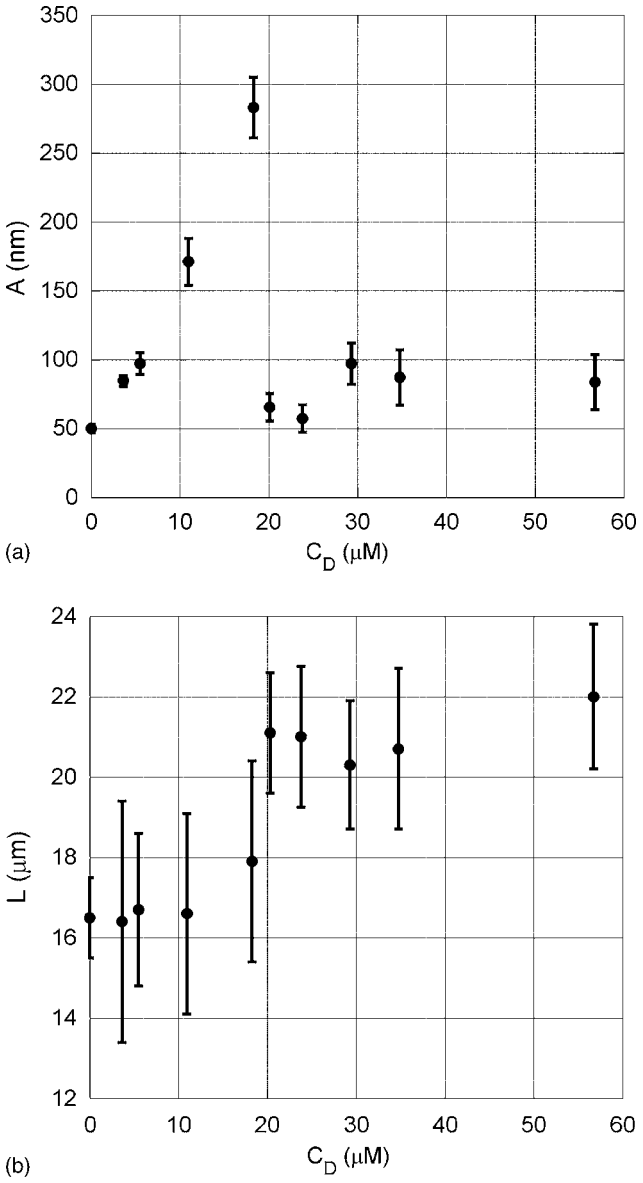

FIG. 2. (a) Persistence length A of DNA-daunomycin complexes as a function of drug concentration for fixed DNA concentration ($C_{bp}=11$ μM). A initially increases with C_D until it reaches a maximum value (~280 nm) at the critical concentration $C_D^{\text{critical}}=18.3$ μM. Then, the persistence length decays abruptly to around 75 nm and remains approximately constant at this value for the concentration range used. (b) Corresponding contour lengths L of the DNA-daunomycin complexes shown in Fig. 2(a) as a function of drug concentration. L increases monotonically with C_D up to the saturation value of 21 ± 1.8 μm.

For comparison purposes, Fig. 3(a) shows three force versus extension curves (normalized by the contour length) for a bare DNA and two different daunomycin concentrations, before and after the transition. The data points in this figure are smoothed out. The purpose of this smoothing process is only to diminish the Brownian fluctuations, and therefore, to make the data easier and clearer to visualize in a plot. This process is done using the smooth routine of the software KALEIDA GRAPH (Synergy Software). We emphasize, however, that all fittings and values for A and L are obtained

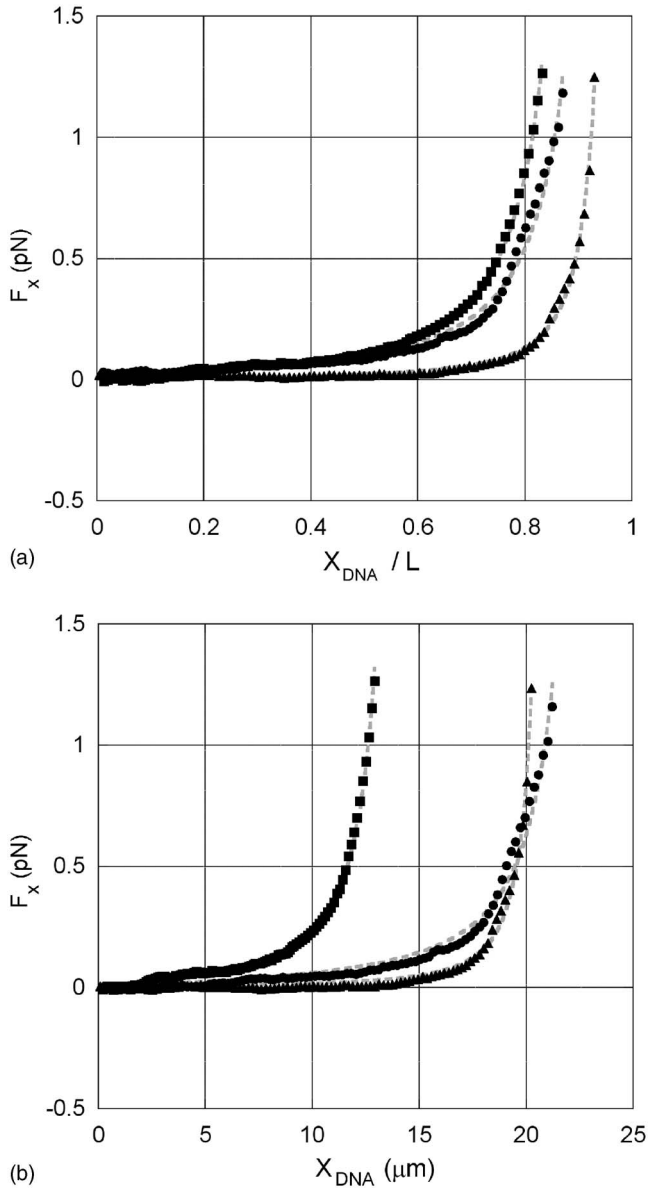

FIG. 3. (a) Force vs extension curves (normalized by the contour length) for a bare DNA molecule and two DNA-daunomycin complexes with different concentrations. The Brownian fluctuations are averaged out for better visualization of the data using a smoothing routine. (b) The same curves of Fig. 3(a) (unscaled). Squares: bare DNA; circles: C_D=20.1 μM (above the critical concentration) and $A \sim 61$ nm; triangles: C_D=18.3 μM and $A \sim 263$ nm. Dashed lines are fittings using Eq. (1).

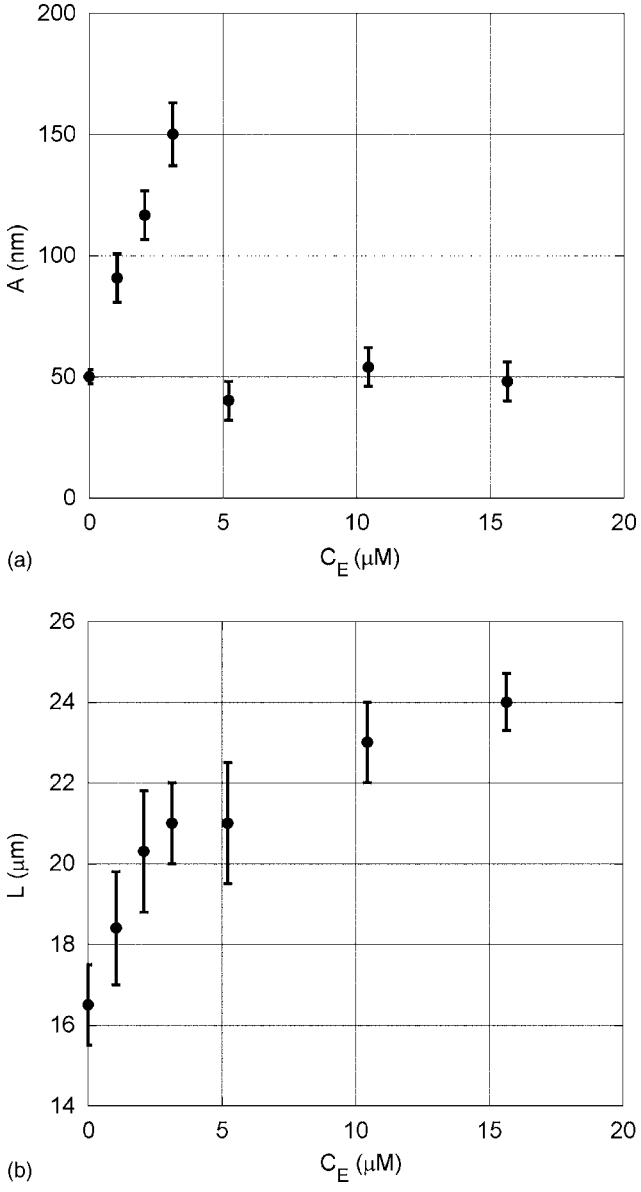

FIG. 4. (a) Persistence length of DNA-EtBr complexes as a function of drug concentration for fixed DNA concentration (C_{bp}=11 μM). In this case, the transition occurs at a lower drug concentration. The maximum value measured for the persistence length of DNA-EtBr complexes is ~150 nm, at the critical concentration $C_E^{critical}$=3.1 μM. (b) Corresponding contour lengths L of the DNA-EtBr complexes shown in Fig. 4(a) as a function of drug concentration. L increases monotonically with C_E up to the saturation value of 23±1 μm.

from the raw data. Figure 3(b) shows the same curves unscaled. In general smoothed data for lower drug concentrations (smaller than the critical concentration) fit better to Eq. (1) than the data for higher drug concentration, as can be seen in Figs. 3(a) and 3(b).

B. Ethidium bromide (EtBr)

The behavior of the persistence length as a function of the drug concentration for DNA-EtBr complexes is very similar to the DNA-daunomycin complexes. The difference is that in this case the transition occurs at a lower drug concentration [see Fig. 4(a)] for the same DNA base pair concentration C_{bp}=11 μM. The maximum value measured for the persistence length of DNA-EtBr complexes is ~150 nm, at the critical concentration $C_E^{critical}$=3.1 μM.

The contour length again increases monotonically from 16.5±1 μm up to the saturation value of 23±1 μm. In Fig. 4(b) we plot the corresponding values of the contour length of the same DNA-drug complexes of Fig. 4(a) as a function of total drug concentration C_E.

Repeating the same calculation for the exclusion parameter of EtBr, which increases the DNA contour length by 0.34 nm per intercalated molecule,[11] we obtain n=2.5, which is in reasonable agreement with the value of 2.01 reported in Ref. 19. In Sec. III C we will show a better estimation for n.

C. Equilibrium binding constants

In our experiments we control the total drug concentration C_T and the total concentration of DNA base pairs C_{bp}. To discuss the elastic properties of the DNA complex formed the important parameter to consider is the ratio r between the concentration of bounded drug (C_b) per concentration of DNA base pairs (C_{bp}). In order to obtain r, the binding of molecules to DNA is analyzed using the neighbor exclusion model.[9] A closed form for this model was obtained by McGhee and von Hippel[20] and can be expressed by the equation

$$\frac{r}{C_f} = K_i(1-nr)\left[\frac{1-nr}{1-(n-1)r}\right]^{n-1} \quad (2)$$

where r is the ratio between the concentration of bounded drug (C_b) per concentration of DNA base pairs (C_{bp}), C_f is the concentration of free drug (not bounded), K_i is the intrinsic binding constant, and n is the exclusion parameter in base pairs. For a more detailed discussion about the neighbor exclusion model, see Ref. 20.

The concentration of free drug (C_f) can be simply related to the concentration of bounded drug (C_b) and the total drug concentration (C_T) through the equation

$$C_T = C_f + C_b. \quad (3)$$

Using Eqs. (2) and (3) with the determined exclusion parameter ($n=3.3$), the intrinsic binding constant reported in Ref. 9 for daunomycin, $K_i = 7\times10^5 M^{-1}$, the critical daunomycin concentration measured in this work ($C_D^{\text{critical}} = 18.3\ \mu$M), and the concentration of DNA base pair used in our experiments ($C_{bp}=11\ \mu$M), we can determine the critical ratio r_c, which we define as the value of r at the abrupt transition for the value of the persistence length. We then obtain the value $r_c=0.248$.

Similarly, for EtBr, we use the parameters $n=2.5$, $K_i = 1.5\times10^5 M^{-1}$,[21] and $C_E^{\text{critical}}=3.1\ \mu$M determined again from the abrupt change in persistence length. We obtain $r_c = 0.131$. In Sec. III D we compare the values obtained for r_c with those reported in the literature for a sedimentation experiment.

It is important to mention that K_i varies with the ionic strength of the solution. The values used here are the values for the ionic concentrations used in our experiments.

To study the variation of the contour length of the DNA-drug complexes when increasing the drug concentration, we plot the total EtBr concentration C_E as a function of the relative increase of the contour length $\Delta L/L_0$ for DNA-EtBr complexes (Fig. 5), where L_0 is the contour length for bare DNA molecules ($16.5\pm1\ \mu$m). Observe that this curve does not exhibit any abrupt changes, indicating that the abrupt change of the persistence length [Fig. 2(a)] is not an artifact.

The relative increase of the contour length ($\Delta L/L_0 \equiv \ell$) can be related to the parameter $r=C_b/C_{bp}$ from Eq. (2). To do this, we write $L_0=N_{bp}\Delta$, where N_{bp} is the number of base pairs and Δ is the distance between two consecutive base pairs. For the bare λ-DNA, we have $N_{bp}=48\,500$ and $\Delta = L_0/N_{bp}=0.34$ nm. When N_b drug molecules intercalate the λ-DNA, the final resulting contour length can be written as

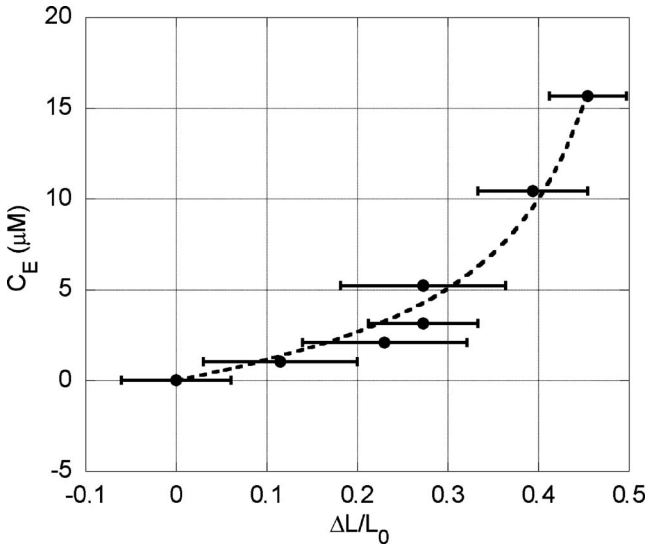

FIG. 5. Total drug concentration C_E as a function of relative increase of the contour length $\Delta L/L_0$ for DNA-EtBr complexes. Fitting this curve to the neighbor exclusion model (dashed line), Eq. (4), we determine the intrinsic binding constant $K_i=(1.4\pm0.4)\times10^5 M^{-1}$ and the exclusion parameter $n=2.35\pm0.15$.

$L=L_0+N_b\delta$, where δ is the increase of the contour length caused by each intercalated molecule. From the literature, we know that $\delta=0.34$ nm for EtBr (Ref. 11) and $\delta=0.31$ nm for daunomycin.[8] We can then write $\ell = N_b\delta/N_{bp}\Delta$. The parameter r can be written as $r=C_b/C_{bp}=N_bM_b/N_{bp}M_{bp}$, where M_b and M_{bp} are, respectively, the molar weight of the drug and of a base pair. Finally, we can write $N_b/N_{bp}=rM_{bp}/M_b$ and $\ell = rM_{bp}\delta/M_b\Delta$. Defining $\gamma = M_{bp}\delta/M_b\Delta$ we write $r=\ell/\gamma$. Using $M_b=394.3$ g/mol for EtBr, $M_b=564$ g/mol for daunomycin, and $M_{bp}=646.4$ g/mol, we determine $\gamma=1.64$ for EtBr and $\gamma=1.04$ for daunomycin.

Using Eqs. (2) and (3) and $r=\ell/\gamma$, one can show that

$$C_T = \frac{C_{bp}}{\gamma}\ell + \frac{\ell(\gamma - n\ell + \ell)^{n-1}}{K_i(\gamma - n\ell)^n}. \quad (4)$$

Since $C_{bp}=11\ \mu$M and $\gamma=1.64$ for EtBr, we fit the data of Fig. 5 to Eq. (4) with two free parameters: K_i and n. From this fit (dashed line in Fig. 5), we determine the intrinsic binding constant K_i and the exclusion parameter n. We have found the values $K_i=(1.4\pm0.4)\times10^5 M^{-1}$ and $n=2.35\pm0.15$ for EtBr, where the error bars are given by the fit. The value determined for K_i is in good agreement with the result from Gaugain *et al.*[21] The value determined for n is in good agreement with the value estimated in Sec. III B, and in reasonable agreement with the value of 2.01 reported in Ref. 19.

For DNA-daunomycin complexes, we find similar results. The values of K_i and n determined from the fit to Eq. (4) are, however, affected by large error bars.

D. Interpretation and discussion of the results

For low drug concentrations, drug intercalation in the DNA molecule increases the rigidity of the complex [see Figs. 2(a) and 4(a)]. This is consistent with the results of Vladescu *et al.*,[22] which shows that EtBr stabilizes the DNA double helix for low drug concentrations. They have per-

formed melting experiments with various EtBr concentrations, from 0 to 2.5 μM, showing that EtBr intercalation stabilizes the DNA double helix in this concentration range. Therefore, we expect an increase of the persistence length of DNA-drug complexes in this low concentration range, in agreement with our data. Figure 4(a) shows this increase for EtBr, and Fig. 2(a) shows similar result for daunomycin.

For high drug concentrations, i.e., above the critical concentration [peak of Figs. 2(a) and 4(a)], the persistence length of the complexes decays abruptly and remains approximately constant in the concentration range studied.

It is well known that intercalation unwinds the DNA double helix.[8] Due to unwinding and above some drug critical concentration, the complexes can have a helix-coil transition, which can cause DNA denaturing as the DNA is stretched, decreasing the persistence length of DNA-drug complexes, as seen in Figs. 2(a) and 4(a). The unwinding angle per intercalated EtBr drug molecule is approximately 1.7 times greater than that for daunomycin intercalation.[8] Therefore, we expect that the transition occurs for EtBr at a lower drug concentration as compared with daunomycin, if the same DNA concentration is used. This is confirmed experimentally in our data of Figs. 2(a) and 4(a). The persistence length of DNA-EtBr complexes increases more rapidly with concentration than the DNA-daunomycin complexes. This behavior reflects the fact that EtBr is the stronger intercalator. The maximum value reached for the persistence length, however, is greater for DNA-daunomycin complexes. This might be explained assuming that the maximum value obtained for the persistence length depends on the competition between two effects caused by the intercalator. While the number of intercalated molecules increases the value of A, the angle unwound by each intercalated molecules may decrease it.

Sedimentation experiments performed with circular DNA as a function of daunomycin and ethidium bromide concentrations display a minimum in the sedimentation coefficient S_{20} at $r_c=0.192$, for daunomycin and $r_c=0.114$ for ethidium bromide.[8] The minimum in the sedimentation coefficient S_{20} is associated with a helix-coil transition, due to unwinding of the DNA double helix by the intercalating drugs.[8] These numbers agree within 15%–30% with the values of r_c determined from our DNA persistence length measurements. This indicates that the abrupt change of the DNA persistence length for both drugs might be also caused by a helix-coil transition due to the unwinding of the DNA double helix as the drugs intercalate into it.

In addition, it is known that EtBr (and also most intercalating drugs) exhibits multimodality at their interaction with DNA.[23,24] The kind of interaction varies with the drug concentration. The abrupt transition shown in Figs. 2(a) and 4(a) might as well be caused by different ways of drug binding to DNA. If this is true, we should see a variation on the persistence length of the complex for concentrations above the critical value. In our data [Figs. 2(a) and 4(a)], the persistence length appears to remain constant in the concentration range used, but perhaps it will not remain at this value if we continue to increase the drug concentration. This may explain the difference observed in our value of the persistence length at this concentration range and some values reported in the literature, while in most cases the ratio C_T/C_{bp} is much higher than our ratio for the critical concentration of the drugs.

The abrupt change in the persistence length observed here depends on the ratio used for the drug concentration and base pair concentration C_T/C_{bp}. In our experiments, we use a constant base pair concentration $C_{\mathrm{bp}}=11$ μM to study the persistence length as a function of total drug concentration. However, if we change C_{bp}, we will get other values for the critical concentrations of each studied drug, because the binding depends on both DNA and drug concentrations.

In the works cited in the Introduction (Sec. I), for example, Smith *et al.*[2] use typical ratios C_T/C_{bp} from 0.403 to 8.07, which are above the critical ratio $C_E^{\mathrm{critical}}/C_{\mathrm{bp}}=0.282$ determined for DNA-EtBr complexes from our data, at the critical concentration. These authors report that EtBr does not modify the elasticity of the DNA molecule, only increasing its contour length by ~40%. Since their C_T/C_{bp} ratio is above the critical one, we can conclude that their results agree with ours because the value obtained for the persistence length of DNA-EtBr complexes above the critical concentration is very close to the value for bare DNA molecules. Also, the increase for the contour length determined from our data is about 39%, in agreement with Ref. 2. Tessmer *et al.*[10] use a C_T/C_{bp} ratio of ~169.5, while Sischka *et al.*[11] use a C_T/C_{bp} ratio of ~1.37 for DNA-EtBr complexes. In these two works, this ratio is well above the value at the critical concentration we measured, therefore, both groups worked at a concentration range above the critical transition. The values obtained for the persistence length in the two works are lower than those for the bare DNA, which suggest that A should decrease if we continue to increase the drug concentration in the region with concentrations greater than the critical one. Our data do not show this effect probably because we use a maximum concentration value of approximately three times the critical one for both drugs. Recently, Vladescu *et al.*[12] reported the value of 5.7 nm for the persistence length of saturated DNA-EtBr complexes. These authors work in a much higher force regime than ours (~100 pN) and use a much higher drug concentration. The results reported by these authors also indicate that A may decrease if we continue to increase the drug concentration above the critical one. Also, the values of A may depend on the force regime used to stretch the DNA molecules. The authors have shown in Ref. 12 that parameters such as the exclusion number n and the binding constant K_i depend on the force used to stretch the molecule. Then, it is reasonable to suppose that A also depend on the force used because the characteristics of the drug intercalation are changed.

In our work, we perform the experiments in a low-force regime (~2–3 pN), i.e., the values reported in our work correspond approximately to the zero-force intercalation situation.

Because most authors uses a C_T/C_{bp} ratio greater than the value determined from our data for the critical concentration, they have missed the transition of the persistence length which we report in this work.

IV. CONCLUSION

We have made systematic measurements of the entropic elasticity variation of a λ-DNA molecule when interacting with two drugs, daunomycin and ethidium bromide, as a function of their concentrations. Our results show that the persistence length of the DNA-drug complexes increases strongly as the drug concentration increases, for low concentrations. Above certain critical drug concentration the persistence length decreases abruptly and remains approximately constant for the range of drug concentrations used above the critical concentration. This behavior is quite similar for both daunomycin and EtBr, as shown in Figs. 2(a) and 4(a). Our results suggests that the abrupt transition observed in the persistence length might be due to a helix-coil transition and denaturing of DNA-drug complexes above the critical concentration, resulting in a decrease of the persistence length. The measured results of the contour length for both DNA-drug complexes fits well to the neighbor exclusion model and do not exhibit any abrupt changes as the drug concentration is varied.

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