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Determining DNA Supercoiling Enthalpy by Isothermal Titration Calorimetry

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Abstract

DNA supercoiling plays a critical role in certain essential DNA transactions, such as DNA replication, recombination, and transcription. For this reason, exploring energetics of DNA supercoiling is fundamentally important for understanding its biological functions. In this paper, using a unique property of DNA intercalators, such as ethidium bromide and daunorubicin, which bind to supercoiled, nicked, and relaxed DNA templates with different DNA-binding enthalpies, we determined DNA supercoiling enthalpy of plasmid pXXZ6, a 4.5 kb plasmid to be about 11.5 kcal/mol per linking number change. This determination allowed us to partition the DNA supercoiling free energy into enthalpic and entropic contributions where the unfavorable DNA supercoiling free energy exclusively originated from the large positive supercoiling enthalpy and was compensated by a large, favorable entropy term ($T \Delta S$).

Graphical Abstract

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Conflict of interest
None declared

Keywords

DNA supercoiling free energy; DNA supercoiling enthalpy; DNA supercoiling entropy; DNA linking number change; isothermal titration calorimetry

1. Introduction

DNA supercoiling is a fundamental property of the DNA double helix [1-3]. For most organisms, DNA is typically negatively supercoiled [1]. The excess energy of negatively supercoiled DNA promotes a number of essential DNA transactions, such as DNA replication, recombination, and transcription [1,2,4-9]. Therefore, analyses of DNA supercoiling free energy are fundamentally important for understanding the physiological functions of DNA supercoiling in cells. Additionally, DNA supercoiling allows cells to compact DNA into a very small volume [1]. In fact, (-) supercoiling coupled with nucleoid-associated proteins, such as HU, H-NS, and FIS, compacts the *E. coli* chromosomal DNA ~1000-fold into the nucleoid of *E. coli* cells [10]. In eukaryotes, the first stage of DNA compaction involves wrapping DNA around a histone octamer core in a left-handed superhelix to form nucleosomes [1], and (-) supercoiling greatly promotes this process [11,12].

Investigations of DNA supercoiling energetics started in the 1970s. The first measurement of DNA supercoiling free energy was carried out by Bauer and Vinograd [13] using the DNA intercalator ethidium bromide, titrated into nicked and negatively supercoiled SV40 DNA. The supercoiling free energy of SV40 DNA was estimated to be ~18 cal/mol per base pair [13]. A few years later, Keller and Wendell demonstrated that agarose gel electrophoresis was able to separate individual DNA topoisomers of closed circular DNA templates [14,15]. Using this method, Depew and Wang [16], and Vinograd and coworkers [17] analyzed the distributions of DNA topoisomers and showed that the DNA supercoiling free energy is a quadratic function of the linking number change (ΔLk). They also formulated an equation to calculate the supercoiling free energy for closed circular DNA molecules between 2 to 10 kb [16]. Although a more recent study demonstrated that the supercoiling free energy was dependent on the experimental conditions and could be varied by more than 50% [18], the formula derived by Depew and Wang is still valid for calculating DNA supercoiling free energy under physiological conditions [16]. These experimental results are also consistent with theoretical analyses of supercoiled DNA molecules, demonstrating that the free energy of negatively supercoiled DNA is quite excessive and can provide substantial driving force when coupled to other reactions [19-22].

So far, there have been a few attempts to determine DNA supercoiling enthalpy which allows the decomposition of the DNA supercoiling free energy into enthalpic and entropic contributions [23-26]. All experimental data demonstrated that the unfavorable supercoiling free energy exclusively originated from the large, positive enthalpy. Intriguingly, these studies also indicate that a favorable, positive entropy is associated with the supercoiling process. For instance, Seidl and Hinz [25] used a microcalorimeter and the relaxation reaction of *E. coli* DNA topoisomerase I to demonstrate that the 220 kcal/mol unfavorable

supercoiling free energy of the 11 kb ColE1 amp RSF2124 plasmid came from the 540 kcal/mol enthalpy and was compensated by a large, favorable entropic contribution ($T \Delta S = 320$ kcal/mol). Since microcalorimetry technology has been improved significantly [27,28] and a rapid procedure was developed to purify a large quantity of *E. coli* DNA topoisomerase I [29], we decided to use a MicroCal VPITC calorimeter in our lab to determine the enthalpic changes associated with DNA supercoiling. To our surprise, we were not able to repeat the procedure developed by Seidl and Hinz [25] due to the complex kinetic behavior of *E. coli* DNA topoisomerase I [29]. We therefore decided to utilize a unique property of the DNA intercalators ethidium bromide and daunorubicin which bind to nicked or relaxed and supercoiled DNA templates with different binding enthalpies to determine the DNA supercoiling enthalpy. The DNA supercoiling enthalpy obtained in this study is substantially larger than that determined by Seidl and Hinz [25].

2. Materials and Methods

2.1. Materials

Ethidium bromide, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride, potassium acetate, and dithiothreitol (DTT) were purchased from Sigma-Aldrich Corp. (St. Louis, MO). Daunorubicin hydrochloride was obtained from Waterstone Technology (Carmel, IN). Magnesium acetate was a product of EM science (Gibbstown, NJ). The EDTA disodium salt was obtained from Fisher Scientific (Pittsburgh, PA). The concentrations of ethidium bromide and daunorubicin were calculated according to their extinction coefficients of $5,600 \text{ M}^{-1}\text{cm}^{-1}$ and $11,500 \text{ M}^{-1}\text{cm}^{-1}$ at 480 nm, respectively. Restriction enzymes, Nt. BbvCI, BamHI, and HindIII were purchased from New England Biolabs, Inc. (Beverly, MA). The negatively supercoiled plasmid DNA pXXZ6 [29] was purified using QIAGEN Plasmid Giga Kits from QIAGEN, Inc. (Valencia, CA). *E. coli* DNA topoisomerase I was purified according to the purification protocol as described in ref [29]. The concentration of *E. coli* DNA topoisomerase I was determined using the extinction coefficient of $98,210 \text{ M}^{-1}\text{cm}^{-1}$ at 280 nm [29]. Recombinant human DNA topoisomerase I was obtained from TopoGen, Inc. (Port Orange, FL).

2.2 Methods

2.2.1. Preparation of relaxed and nicked plasmid DNA pXXZ6—The relaxed plasmid DNA pXXZ6 was prepared in the relaxation buffer containing 20 mM Tris-acetate (pH 7.9), 50 mM KAc, 6 mM $\text{Mg}(\text{Ac})_2$, 1 mM DTT, 0.1 $\mu\text{g/ml}$ BSA, 0.125 mg/ml negatively supercoiled plasmid pXXZ6, and 140 nM of *E. coli* DNA topoisomerase I. After incubation at 37 °C for 30 min, the relaxation reaction was stopped by extraction with an equal volume of phenol. For the preparation of 0.15 mM relaxed plasmid DNA pXXZ6, the DNA sample after phenol extraction was dialyzed extensively against 1×BPES (6 mM Na_2HPO_4 , 2 mM NaH_2PO_4 , 1 mM EDTA, 185 mM NaCl, and pH 7.1) at 4°C. To prepare 1 mM of relaxed plasmid DNA pXXZ6, the DNA sample after phenol extraction was first dialyzed against 1×BPE (6 mM Na_2HPO_4 , 2 mM NaH_2PO_4 , 1 mM EDTA, and pH 7.1) at 4°C and then dried in a lyophilizer. The freeze-dried DNA sample was re-dissolved in 1×BPES and dialyzed against 1×BPES at 4°C for the following ITC experiments.

The nicked pXXZ6 was prepared in a buffer containing 20 mM Tris-HCl (pH 7.9), 50 mM NaCl, 10 mM MgCl₂, 1 mM DTT, 0.1 µg/ml BSA, 0.1 mg/ml negatively supercoiled plasmid pXXZ6, and 150 units/ml Nt. BbvCI. After incubation at 37°C for 2 hours, the reaction was stopped by extraction with an equal volume of phenol. For the preparation of 0.15 mM nicked plasmid DNA pXXZ6, the DNA sample after phenol extraction was dialyzed extensively against 1×BPES at 4°C. To prepare 1 mM nicked plasmid DNA pXXZ6, the DNA sample after phenol extraction was firstly dialyzed against 1×BPE at 4°C, and then dried in a lyophilizer. The freeze-dried DNA sample was re-dissolved in 1×BPES and dialyzed against 1×BPES at 4°C. The DNA concentration of plasmid pXXZ6 was estimated using the formula of 1 OD₂₆₀ = 0.15 mM (bp).

2.2.2. Isothermal titration calorimetry (ITC)—ITC experiments were carried out using a VP-ITC titration calorimeter (MicroCal, Inc., Northampton, MA) interfaced to a personal computer. Origin 7.0, supplied by the manufacturer was used for data acquisition. For a typical ITC experiment, the titration was set up so that 10 µL of a 1 mM intercalator, i.e., ethidium bromide or daunorubicin, was injected every 300 seconds, up to a total of 29 injections, into a DNA sample (1.44 ml of 150 µM(bp)) in the sample cell or 5 µL of 1 mM intercalating reagent was injected every 300 seconds, up to a total of 25 injections, into a DNA sample (1.44 ml of 1 mM(bp)) in the sample cell. The heat liberated or absorbed is observed as a peak corresponding to the power required to keep the sample and reference cells at identical temperatures. The peak produced during the injection is converted to heat output by integration and corrected for cell volume and sample concentration. Control experiments were also carried out to determine the contribution of the heats of dilution arising from (1) the DNA intercalators into buffer (Fig. S1) and (2) buffer into DNA. The net enthalpy for the titration reaction was determined by subtraction of the component heats of dilution.

2.2.3. Determining supercoiling density of plasmid pXXZ6 and calculating its supercoiling free energy—3 µg of supercoiled plasmid pXXZ6 was relaxed by human DNA topoisomerase I in the presence of various concentrations of ethidium bromide at 37°C in 20 mM Tris-acetate (pH 7.9), 50 mM KAc, 10 mM Mg(Ac)₂, and 1 mM DTT for one hour. Subsequently, the relaxation reaction was stopped by addition of an equal volume of phenol. The topological status of each DNA preparation was analyzed by electrophoresis in a 1% agarose gel in 1×TAE buffer (40 mM Tris-acetate (pH 7.8) and 1 mM EDTA) containing different concentrations of chloroquine. After electrophoresis, agarose gels were stained with ethidium bromide, destained, and photographed under UV light. The DNA linking number change (ΔLk) was determined by analyzing the distributions of the topoisomers in these gel images and the supercoiling density (σ) was calculated using the following equation:

$$\sigma = \frac{\Delta Lk}{Lk^0} = \frac{Lk - Lk^0}{Lk^0} \quad (1)$$

where Lk^0 and Lk represent the DNA linking number for the relaxed and the supercoiled DNA, respectively.

The DNA supercoiling free energy for circular plasmid DNA templates was calculated according to the following equation [16]:

$$\Delta G_{\Delta Lk} = \frac{1200RT(\Delta Lk)^2}{N} \quad (2)$$

where $\Delta G_{\Delta Lk}$ is the DNA supercoiling free energy at the ΔLk , R is the gas constant, T is temperature in Kelvin, and N is the number of base pairs for the plasmid DNA template. ΔS was calculated according to:

$$\Delta G_{\Delta Lk} = \Delta H_{\Delta Lk} - T\Delta S_{\Delta Lk} \quad (3)$$

where $\Delta H_{\Delta Lk}$ and $\Delta S_{\Delta Lk}$ are the DNA supercoiling enthalpy and entropy at the ΔLk , respectively.

3. Results and Discussion

Initially we tried to use ITC experiments to determine the kinetic parameters of *E. coli* DNA topoisomerase I. Our control experiments showed that our VP-ITC calorimeter was capable of deriving K_M and k_{cat} of trypsin from the ITC experiments (Fig. S2). However, due to the “atypical” and complex kinetic mechanism of *E. coli* DNA topoisomerase I, our ITC experiments failed to yield K_M and k_{cat} for this enzyme. Because we already purified a large quantity of *E. coli* DNA topoisomerase I [29], we tried to repeat the calorimetry experiments carried out by Seidl and Hinz [25] where it was demonstrated that the unfavorable supercoiling free energy exclusively comes from the positive enthalpy associated with the formation of the negatively supercoiled plasmids. However, after many trials, we were not able to obtain a reliable DNA supercoiling enthalpy from the ITC experiments using the relaxation assay of *E. coli* DNA topoisomerase I. In this case, we sought to use an alternative strategy to measure the DNA supercoiling enthalpy. Since DNA intercalators, such as ethidium bromide and daunorubicin, unwind the DNA templates upon binding to circular plasmid DNA ([30]; Fig. 1), it is possible to derive the DNA supercoiling enthalpy from the difference between the DNA-binding enthalpy for the DNA intercalator binding to the supercoiled plasmid DNA and the nicked or relaxed plasmid DNA using the following equations:

$$\Delta H_s = (\Delta H_{Nk} - \Delta H_{Sc}) \times \frac{360}{\beta} \quad (4)$$

or

$$\Delta H_s = (\Delta H_{Rx} - \Delta H_{Sc}) \times \frac{360}{\beta} \quad (5)$$

where ΔH_s , ΔH_{Sc} , ΔH_{Nk} , and ΔH_{Rx} represent the DNA supercoiling enthalpy per linking number change, the DNA-binding enthalpy for the intercalator binding to the supercoiled plasmid DNA, the DNA-binding enthalpy for the intercalator binding to the nicked plasmid DNA, and the DNA-binding enthalpy for the intercalator binding to the relaxed plasmid DNA, respectively. β is the DNA unwinding angle of the DNA intercalator. Ethidium

bromide was used as the first DNA intercalator for our ITC experiments. Fig. 2 shows results where ethidium bromide was titrated into a large excess (1 mM(bp)) of either the supercoiled (Fig. 2A), the nicked (Fig. 2B), or the relaxed (Fig. 2C) plasmid pXXZ6 (the status of the supercoiled, relaxed, and nicked plasmid pXXZ6 is shown in Fig. S3). In this case, all added ethidium bromide was bound to DNA after each injection. The use of a large excess DNA substrate should also reduce experimental deviations. The DNA-binding enthalpy for ethidium bromide binding to the supercoiled and the nicked plasmid DNA pXXZ6 was estimated to be $-7,945 \pm 42$ cal/mol and $-7,120 \pm 52$ cal/mol, respectively (Table 1). The enthalpy difference between ethidium bromide binding to the supercoiled and the nicked DNA was calculated to be 825 ± 94 cal/mol (Table 1 and Fig. 3A). Since each ethidium bromide molecule unwinds DNA by about 26 degrees [31], the DNA supercoiling enthalpy (ΔH_S) should equal $11,423 \pm 1,302$ cal/mol per linking number change (equation 4) if we assume no additional enthalpy change was associated with the difference between ethidium bromide binding to the supercoiled and the nicked DNA molecules (Table 1). We also estimated the DNA supercoiling enthalpy (ΔH_S) by comparing the difference between ethidium bromide binding to the supercoiled and the relaxed plasmid DNA pXXZ6 (Fig. 2). The enthalpy changes for ethidium bromide binding to the supercoiled and the relaxed plasmid DNA pXXZ6 were estimated to be $-7,945 \pm 42$ cal/mol and $-7,049 \pm 52$ cal/mol, respectively (Fig. 2 and Table 1). The enthalpy difference between ethidium bromide binding to the supercoiled and the relaxed DNA was calculated to be 898 ± 253 cal/mol which is almost equivalent to the enthalpy difference between ethidium bromide binding to the supercoiled and the nicked DNA (Table 1 and Fig. 3B). In this case, the DNA supercoiling enthalpy (ΔH_S) was calculated to be $12,433$ cal/mol per linking number change using equation 5 (Table 1). The DNA supercoiling enthalpy determined here is consistent with the results obtained from a previous study on the unwinding of dsDNA by dehydration [24] where the enthalpy value for unwinding a turn of DNA double helix was estimated to be ~ 12.2 kcal/mol. However, our DNA supercoiling enthalpy is substantially larger than the one estimated from the calorimetric measurement of the DNA relaxation by *E. coli* DNA topoisomerase I [25]. A possible reason for the difference is that the relaxation reaction of *E. coli* DNA topoisomerase I is a relatively slow process [29] and it is difficult to obtain a good baseline for integration of the enthalpy. Therefore, it will result in a large error in the DNA supercoiling enthalpy. Another possibility is that the enthalpy obtained from the relaxation reaction also contains a contribution from *E. coli* DNA topoisomerase I binding to the supercoiled DNA template. In this case, the enthalpy obtained from the calorimetric measurement of the DNA relaxation reaction by *E. coli* DNA topoisomerase I also has a contribution from *E. coli* DNA topoisomerase I binding to the supercoiled DNA template, which causes the DNA supercoiling enthalpy to deviate from the values obtained by using other techniques. A third reason for the difference is that the experimental conditions in these two measurements are quite different. We used 200 mM Na^+ at 25°C in our ITC experiments while Seidle and Hinz used a relatively low salt buffer solution at 37°C for the DNA topoisomerase I relaxation assays [25]. Nevertheless, as mentioned above, we were not able to repeat the relaxation assays using our VP-ITC calorimeter.

In this study, we also carried out another type of ITC experiments in which a series of 10 μl of 1 mM ethidium bromide were titrated into 0.15 mM (bp) of the supercoiled, the nicked

and the relaxed plasmid DNA solution. Under this condition, all DNA binding sites on DNA lattice will be titrated up by ethidium bromide. Fig. 4 shows the results of these ITC experiments. The DNA binding enthalpy for ethidium bromide binding to the supercoiled, nicked, and relaxed plasmid DNA pXXZ6 were estimated to be $-7,439 \pm 51$, $-6,670 \pm 66$, and $-6,601 \pm 45$ cal/mol, respectively (Table 1). These values are consistent with those shown in Fig. 2. Interestingly, the enthalpy differences between the supercoiled and the nicked plasmid DNA are quite dramatic over the entire course of the titration experiment. They are initially negative, then positive, and finally close to zero (Fig. 5A). We believe that this ITC binding curve is a result of ethidium bromide unwinding the DNA double helix upon binding to the negatively supercoiled DNA (Fig. S4A). In this case, the twist is reduced, the writhe is increased, and therefore the DNA templates become more relaxed. Further addition of ethidium bromide leads to the production of positively supercoiled plasmid DNA (Fig. S4A). These results suggest that a positive enthalpy is associated with the formation of the negative superhelical turns and a negative enthalpy may be involved in the generation of the positive superhelical turns. For the enthalpy differences between ethidium bromide binding to negatively supercoiled and relaxed DNA templates, we only observed a “normal” titration curve (Fig. 5C) where no positive enthalpy was involved. The main reason is that the titration of ethidium bromide into the relaxed plasmid DNA templates also leads to the production of the positively supercoiled DNA (Fig. S4C). Regardless, the DNA supercoiling enthalpy (H_S) was calculated to be $10,648 \pm 1,620$ or $11,603 \pm 1,329$ cal/mol, which is consistent with the values determined above.

Next we used another DNA intercalator daunorubicin to estimate the DNA supercoiling enthalpy by isothermal titration calorimetry. Since the DNA unwinding angle of daunorubicin is 11 degrees [32], the enthalpy difference between daunorubicin binding to the negatively supercoiled and the nicked or the relaxed DNA templates should be half of the value for ethidium bromide binding to these DNA templates. Our results are shown in Fig. S5, S6, and S7. For the ITC titration experiments using a large excess (1 mM(bp)) of either the supercoiled (Fig. S5A) or the nicked (Fig. S5B) or the relaxed (Fig. S5C) pXXZ6, the DNA-binding enthalpies of daunorubicin were estimated to be $-8,538 \pm 85$, $-8,030 \pm 52$, and $-8,086 \pm 92$ cal/mol, respectively (Table 1). The enthalpy differences between daunorubicin binding to the supercoiled and the nicked or the relaxed DNA were calculated to be 452 ± 134 and 508 ± 177 cal/mol, respectively (Table 1). Using equations 4 and 5, the DNA supercoiling enthalpy (H_S) was calculated to be $14,792 \pm 4,386$ or $16,625 \pm 5,793$ cal/mol per turn of DNA supercoil, values substantially larger than those determined by using ethidium bromide as the DNA intercalator. One possible reason for the difference is that daunorubicin strongly self-associates in aqueous solution at concentration greater than $10 \mu\text{M}$ [33]. In this case, the heat of dilution is quite large and non-constant (Fig. S1B) and may significantly affect the accuracy of the determination. Another possible reason is that daunorubicin has a sugar ring located the minor groove of DNA of daunorubicin-DNA complexes [34]. This binding property may yield a different binding enthalpy to supercoiled, relaxed and nicked plasmid DNA templates and therefore also affect the accuracy of the determination. Because of these concerns, for further analyses, we decided to use an average value of the DNA supercoiling enthalpy derived only from the ITC experiments using ethidium bromide as the DNA intercalator, i.e., $H_S = 11.5$ kcal/mol. For the ITC titration

experiments using 0.15 mM (bp) of the supercoiled and the relaxed plasmid DNA solution, the DNA supercoiling enthalpy was calculated to be $15,643 \pm 2,749$ cal/mol, which is in the same range of the DNA supercoiling enthalpy determined using a large excess of DNA substrate.

With the knowledge of the DNA supercoiling enthalpy, we now are able to partition the DNA supercoiling free energy (G_{Lk}) to H_{Lk} and $T S_{Lk}$. For supercoiled plasmid pXXZ6 used in this study, we determined the supercoiling density of the plasmid to be -0.061 . In this case, Lk equals -26 . Using equation 2, we calculated its supercoiling free energy to be 107 kcal/mol at 25°C. As demonstrated above (Table 1), the average DNA supercoiling enthalpy per linking number change is ~ 11.5 kcal/mol. For supercoiled pXXZ6 with Lk of -26 , H equals 299 kcal/mol and $T S$ equals 192 kcal/mol. This analysis indicates that the unfavorable supercoiling free energy stems exclusively from the large unfavorable DNA supercoiling enthalpy (Fig. 6 and Table 2), which is consistent with previously published results [19,20]. Since DNA supercoiling deforms the DNA double helix, the large positive supercoiling enthalpy is not totally unexpected [19]. Regardless, the large, positive supercoiling enthalpy is also compensated by a large, favorable entropy term. As suggested by Vologodskii and Cozzarelli [19], the large, favorable entropy may come from releasing the bound ions and water molecules upon supercoiling. Additionally, as Delrow pointed out, part of the favorable entropy may originate from the temperature-dependent torsional rigidities of DNA molecules [35]. Nevertheless, further studies are needed to determine the origin of the large, favorable supercoiling entropy.

4. Conclusions

By utilizing a unique property of DNA intercalators in which they are able to unwind the DNA double helix upon binding to circular plasmid DNA templates, we determined the DNA supercoiling enthalpy to be about 11.5 kcal/mol per linking number change using isothermal titration calorimetry. These values are in good agreement with the results obtained from a previous study on the unwinding of dsDNA by dehydration [24]. We also demonstrated that positive enthalpy is associated with the formation of negative supercoils and negative enthalpy may be involved in the generation of positive supercoils. Additionally, we showed that the unfavorable DNA supercoiling free energy exclusively came from the large positive supercoiling enthalpy which was compensated by a large, favorable entropy term ($T S$).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ITC	isothermal titration calorimetry
EB	ethidium bromide
Sc	supercoiled
Nk	nicked
Rx	relaxed

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- DNA intercalators, such as ethidium bromide and daunorubicin, bind to supercoiled, nicked, and relaxed DNA templates with different DNA-binding enthalpies.
- DNA supercoiling enthalpy of plasmid pXXZ6, a 4.5 kb plasmid was determined to be about 12 kcal/mol per linking number change.
- The unfavorable DNA supercoiling free energy exclusively originated from the large positive supercoiling enthalpy and was compensated by a large, favorable entropy term (TDS).

Figure 1.

(A) The plasmid map of pXXZ6. Restriction enzyme recognition sites of BamHI, HindIII, and Nt.BbvCI are shown. For supercoiled pXXZ6 used in this study, the supercoiling density was determined to be -0.061 . (B) DNA intercalator ethidium bromide binds to a negatively supercoiled DNA template and causes DNA unwinding. H_s represents the DNA-binding enthalpy for ethidium bromide binding to the supercoiled plasmid DNA. (C) DNA intercalator ethidium bromide binds to a nicked DNA template. H_{Nk} represents the DNA-binding enthalpy for ethidium bromide binding to the nicked plasmid DNA.

Figure 2.

Sample raw data from isothermal titration calorimetry experiments for the titration of ethidium bromide into (A) supercoiled, (B) nicked, and (C) relaxed plasmid pXXZ6 at 25°C in 1×BPES buffer. Top, each peak corresponds to the heat generated by injection of an aliquot of 5 μ L of ethidium bromide (1 mM) into DNA solution (1.44 ml of 1.0 mM (bp)). Bottom, binding isotherm by integration with respect to time.

Figure 3.

Distribution of the DNA supercoiling enthalpy (ΔH_S) calculated from (A) the enthalpy difference between ethidium bromide binding to supercoiled and nicked pXXZ6 (Table 1) according to equation 4 and (B) the enthalpy difference between ethidium bromide binding to supercoiled and relaxed pXXZ6 (Table 1) according to equation 5.

Figure 4.

Sample raw data from isothermal titration calorimetry experiments for the titration of ethidium bromide into (A) supercoiled, (B) nicked, and (C) relaxed plasmid pXXZ6 at 25°C in 1×BPES buffer. Top, each peak corresponds to the heat generated by injection of an aliquot of 5 µL of ethidium bromide (1 mM) into DNA solution (1.44 ml of 0.15 mM (bp)). Bottom, binding isotherm by integration with respect to time.

Figure 5.
The enthalpy difference between ethidium bromide binding to (A) supercoiled and nicked or (B) supercoiled and relaxed plasmid pXXZ6 as a function of molar ratio of ethidium bromide to DNA. The enthalpy values were calculated according to ITC data shown in Fig. 4.

Figure 6.
Thermodynamic profiles of supercoiled plasmid pXXZ6 with a σ of -0.061 at 25°C in $1\times\text{BPES}$ buffer.

DNA-binding Enthalpy for DNA intercalators ethidium bromide and daunorubicin binding to supercoiled (Sc), relaxed (Rx), and nicked (Nk) plasmid DNA pXXZ6

Table 1

	Ethidium Bromide			Daunorubicin		
	H ^d (cal/mol)	H ^b (cal/mol)	H ^c (cal/mol)	H ^d (cal/mol)	H ^b (cal/mol)	H ^c (cal/mol)
Sc pXXZ6	-7,945 ± 42 ^d	—	—	-8,538 ± 85 ^d	—	—
	-7,439 ± 51 ^e	—	—	-5,432 ± 44 ^e	—	—
Rx pXXZ6	-7,049 ± 211 ^d	896 ± 253	12,433 ± 3,510	-8,086 ± 49 ^d	452 ± 134	14,793 ± 4,386
	-6,601 ± 45 ^e	838 ± 96	11,603 ± 1,329	-4,954 ± 40 ^e	478 ± 84	15,643 ± 2,749
Nk pXXZ6	-7,120 ± 52 ^d	825 ± 94	11,423 ± 1,302	-8,030 ± 92 ^d	508 ± 177	16,625 ± 5,793
	-6,670 ± 66 ^e	769 ± 117	10,648 ± 1,620	—	—	—

^a H represents DNA-binding enthalpy for ethidium bromide or daunorubicin binding to Sc, Rx, and Nk pXXZ6 as determined by ITC.

^b H is the enthalpy difference between ethidium bromide or daunorubicin binding to Sc and Rx pXXZ6 or Sc and Nk pXXZ6.

^c Hs represents the DNA supercoiling enthalpy as calculated by equation $Hs = H(360/\beta)$ where β is the DNA unwinding angle of the DNA intercalators.

^d The DNA-binding enthalpy was obtained from titrating the intercalators into 1 mM of plasmid pXXZ6 in 1×BPES.

^e The DNA-binding enthalpy was obtained from titrating the intercalators into 0.15 mM of plasmid pXXZ6 in 1×BPES. The values with calculated standard deviations are the average of at least three independent determinations.

Table 2

Thermodynamic parameters for DNA supercoiling

Size of the plasmid (bp)	G _S (kcal/mol)	H _S (kcal/mol)	T S _S (kcal/mol)	Source
4,483	107 ^a	299 ^a	192 ^a	This work
	4.1 ^b	11.5 ^b	7.4 ^b	
11,000	220 ^a	540 ^a	320 ^a	Ref [25]
	3.7 ^b	9.0 ^b	5.3 ^b	
Various DNA molecules	-	-	-	Ref [24]
	-	12.2 ^b	-	

G_S, H_S, and T S_S represent supercoiling free energy, enthalpy, and entropy contribution, respectively.

^aThese values are calculated for thermodynamic quantities per plasmid.

^bThese values are calculated for thermodynamic quantities per linking number change.