Thermodynamic Parameters Are Sequence-dependent for the
Supercoil-induced B to Z Transition in Recombinant Plasmids*

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The enthalpy and entropy changes which contribute to the thermodynamics of the B to Z transition were determined for three recombinant plasmids containing a (dC-dC)₁₅ tract and for a plasmid containing a pair of (dT-dG)₂₀ regions. For each base pair which adopts a left-handed conformation in the plasmids with (dC-dG)₁₅ sequences, the ΔHₓ and ΔSₓ values are -2.1 kcal/mol bp and -8.8 cal/K-mol bp, respectively. In the plasmid containing the (dT-dG)₂₀ tracts, however, the ΔHₓ and ΔSₓ values are 0.58 kcal/mol bp and -0.76 cal/K-mol bp, respectively. These determinations show that for each B-Z junction that forms in the plasmids containing the (dC-dG), the enthalpy and entropy changes are 24 kcal/mol junction and 65 cal/K-mol junction, whereas for the (dT-dG) plasmid, the enthalpy and entropy changes are -1.8 kcal/mol junction and -22 cal/K-mol junction, respectively. Those values for the enthalpy and entropy changes for the formation of a BZ junction in (dC-dG) and (dT-dG) plasmids suggest that the properties and possibly the structures of the junctions are different. Calculations using the enthalpy and entropy changes determined in this study reveal that the B to Z transition in plasmids containing (dC-dG) blocks are more temperature-dependent than the transitions in plasmids with (dT-dG) blocks. Surprisingly, at temperatures above 60 °C, calculations indicate that the B to Z transitions in (dT-dG) plasmids should be energetically favored over that transition in (dC-dG) plasmids.

Early studies on (dG-dC)ₓ-(dG-dC)ₓ (1, 2) revealed the fundamental thermodynamic and kinetic properties (3) of the salt-induced conformational transition prior to our realization of right-handed to left-handed structural characteristics (4-7). The B to Z transition for this polymer in high molarity salt solutions was an entropically governed process (3). Free energy changes associated with the B to Z transition in plasmids containing (dC-dG) blocks are difficult to quantify. However, there are potential problems using proteins which may influence equilibria to investigate the thermodynamics of that transition. Hence, the most reliable method to monitor the energetics of the B to Z transition in plasmids is supercoil relaxation.

Although one-dimensional electrophoresis provides information on the relaxation of supercoils correlating with the right- to left-handed transition (6, 13-15), data on individual topoisomer distributions are difficult to obtain. However, two-dimensional gel electrophoresis separates the positively and negatively supercoiled topoisomers and, furthermore, permits quantitation (23, 24). Moreover, the data obtained from two-dimensional gels can be fitted to a statistical mechanical model to obtain free energy values for (a) each base pair in a potential B-Z junction which adopt either a B-DNA or Z-DNA structure, and (b) for a free energy of the junction (24-26). Subsequently, two-dimensional gel electrophoresis has enabled studies on the formation of left-handed DNA in other types of sequences (10, 17, 19-21, 27, 28). However, none of the studies on recombinant plasmids which contain left-handed DNA have investigated the influence of temperature on the B to Z transitions.

This report uses two-dimensional gel electrophoresis to determine the effect of temperature on the free energy of the B to Z transition in recombinant plasmids containing both (dC-dG) and (dT-dG) sequences. A statistical mechanical model was employed. We then calculated the enthalpy and entropy changes associated with the B to Z transitions.

**MATERIALS AND METHODS**

Enzymes—Polymerase chain reaction was purchased from P-L Biochemicals. T4 DNA ligase and Klenow fragment DNA polymerase were purchased from Boehringer Mannheim. Restriction enzymes were purchased from Bethesda Research Laboratories, Boehringer Mannheim, or New England Biolabs and used as recommended.

Synthesis of Oligonucleotides—Two deoxynucleotides with sequences of GATCC(TG)₁₀ and AATT(CA)₁₀ were chemically synthesized using an Applied Biosystems model 380A DNA synthesizer which employs phosphoramidite technology (29, 30). The oligonucleotide products were loaded onto a 12% polyacrylamide denaturing gel to separate the reaction products. The bands containing the oligonucleotides of interest were excised from the gel and allowed to elute overnight from the gel slices. The oligomers were further purified using a SEP-PAK cartridge (Waters Associates). At least 5 μg of each strand was obtained using this procedure. The strands of the

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two oligomers were annealed to generate a 42-bp fragment with EcoRI and BamHI sticky ends by mixing 5 µg of each strand in 19 µl of 15 mM Tris-HCl (pH 7.6), 6 mM NaCl, 6 mM MgCl₂, 0.2 mM dithiothreitol at 65°C for 10 min followed by cooling to room temperature.

Plasmids—pRW756 is a 4144-bp plasmid with a (dC-dG)₁ block and a 95-bp fragment containing the lac operator region of Escherichia coli integrated between the EcoRI and BamHI sites of pBR322. The construction of pRW756 was described previously (31). pRW58 and pRW59 are linking number mutants of pRW756 and were constructed by linearizing pRW756 with Avai or NdeI. The 5' phosphate sticky ends of the linearized pRW756 (2.0 µg) were filled in using Klenow fragment DNA polymerase in 50 mM Tris-HCl (pH 8.0), 10 mM MgSO₄, 0.1 mM dithiothreitol, and 0.8 mM dNTPs. These filled-in plasmids (1.0 µg) were ligated with 74 DNA ligase (2 units) at room temperature overnight in 20 µl containing 50 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, 10 mM dithiothreitol, 1 mM spermidine, and 1 mM ATP. These ligation mixtures were then cut again with either Avai or NdeI. The re-cut ligation reactions were then transformed into E. coli HB101, and the transformed cells were plated onto TYE-ampicillin agar plates. Colonies were screened for the plasmids by growing 10 ml cultures in L broth.

pRW155 was constructed using the 42-bp fragment (dT-dG)₁₆ with EcoRI sticky ends and a 95-bp fragment with EcoRI sticky ends (32), (gift of Dr. W. T. Hsieh) containing the lac operator region of E. coli. The 42-bp fragment was reacted with polynucleotide kinase (15 units) in 50 mM Tris (pH 7.6), 40 mM MgCl₂, 5 mM β-mercaptoethanol, and 0.2 mM ATP at 37°C for 40 min. The reaction was stopped by heat inactivating the enzyme at 65°C for 15 min. The ligation mixture was digested with BamHI to generate molecules which contained two 42-bp fragments at the end and one or more 95-bp fragments in the middle of the molecule. This product was then ligated in the presence of the pRW461 (gift of Dr. W. T. Hsieh, this laboratory) which was linearized with BamHI and transformed into E. coli HB101, pRW461 is nearly identical to pBR322 except that the EcoRI site was eliminated by filling in that site with Klenow fragment DNA polymerase. The colonies were selected for recombinant plasmids containing the lac operator fragment using plates containing 5-bromo-4-chloro-3-indoyl-β-D-galactoside (x-gal) and ampicillin. The blue colonies were grown in 10 ml cultures and screened for insert size by restriction enzyme digest. The sequences of these plasmids (Fig. 1) were verified by either M13 dyeoxy chain termination or Maxam and Gilbert sequence determinations.

Plasmids were isolated from 1 to 4 liter growths in M9 medium with chloramphenicol amplification. The plasmids were isolated as described elsewhere.² The growths yielded 1.5–2.5 mg of plasmid/liter.

Topoisomer Populations—Ten separate topoisomer populations were prepared as described (33). The populations were analyzed using electrophoresis. These 10 topoisomer populations were then mixed for two-dimensional gel electrophoresis.

Electrophoresis—Agarose gels (1.2–1.5%) (agarose purchased from IBI) were run at fixed temperatures for 48 h at 1.7 V/cm in 80 mM Tris phosphate, 2 mM EDTA (TPE buffer). Temperature was maintained at ±1°C by conducting electrophoresis (a) in constant temperature rooms, (b) in a gel box with a circulating water plate (International Biotechnologies, Inc. model HRH), or (c) in a constant temperature water bath. Temperature on the surface of the gel was measured using a Cole-Farmer Digital Thermometer with a subsensible probe. Following electrophoresis in the first dimension, the gel section containing the topoisomers for electrophoresis in the second dimension was excited and stained in 4–8 µM chloroquine phosphate in TPE buffer for 1 h. The second dimension was electrophoresed at a 90° angle to the first dimension for 48 h at 1.7 V/cm in TPE buffer containing 4–8 µM chloroquine phosphate.

Data Analysis—The data was fit to a statistical mechanical model using a nonlinear least squares program with the following equation (24):

\[ \Delta T w = K(a - \alpha_2) + K(a - \alpha_1) \exp(-2T_0/RT) + \sum_{i=1}^{N} a_i \exp(-2T_0/RT) \]

where \( \Delta T w \) = average difference in twist, \( a = \exp(-2T_0/RT) \), \( s = \exp(-2T_0/RT) \), \( K = 1100RT/N, R = \text{gas constant}, T = \text{absolute temperature} \) (Kelvin), \( N = \text{number of} \) (dC-dG) units capable of adopting Z-DNA conformation, \( n = \text{number of} \) (dC-dG) units less than or equal to \( n \), \( N = \text{number of base pairs in the plasmid}, \alpha = \text{linking difference}, \alpha_1 + 2b = \text{total helical twist difference}, a = -2(1/10.5 + 1/12), b = \text{change in twist from junction}. In this treatment, the nonlinear least squares value fit is to three independent parameters: \( \Delta T w_0, \Delta T w_{100}, \text{and} \ b \).

This method is analogous to the "zipper model" for DNA thermal melting. The denominator in Equation 1 represents the partition function of the system which describes the distribution of the energy in the system using Boltzmann statistics. The major barrier to the formation of Z-DNA in this model is the formation of the junctions between B- and Z-DNA whose free energy is represented by \( \Delta G_{Z-DNA} \). Following the formation of the junction, however, it is relatively easy for each base pair to adopt a Z-DNA structure with a free energy of formation \( \Delta G_{Z-DNA} \). This model also includes the possibility that the twist at the junction region can change when Z-DNA is formed.

RESULTS

Effect of Temperature on Supercoil-induced Relaxation of pRW756 and pRW155—The extent of negative supercoil relaxation associated with the formation of left-handed DNA is particularly diagnostic, since approximately two turns of su-

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1. The abbreviation used is: bp, base pairs.
2. H. R. Bergen, M. J. Losman, T. O'Connor, W. Zacharias, J. E. Larson, R. D. Wells, and W. J. Koopman, manuscript submitted.
perihelix are relaxed for every one turn of left-handed Z-DNA formed. This phenomenon was first demonstrated by electrophoresis in agarose gels at high NaCl concentrations (6, 15). Subsequent studies used one-dimensional electrophoresis on agarose gels as well as nuclease sensitivity to reveal the formation of left-handed DNA. Two-dimensional gel electrophoresis, however, provides much more sensitivity for the study of structural transitions that are related to superhelical density. The first dimension of the electrophoresis is a normal agarose gel which separates topoisomers. The second dimension is conducted in the presence of an intercalating agent (chloroquine) which enhances the resolution by unwinding the DNA and therefore changing the mobility of the topoisomers. This reduction in negative supercoiling reverses the B to Z transition in the second dimension and, therefore, removes the relaxation due to Z-DNA. This removal of Z-DNA is manifested as a discontinuity in the smooth topoisomer pattern in Fig. 2A ($\tau \approx -14$ to $-15$). The mobility of the topoisomers observed in this region are related to the free energy change of the B to Z transition. In this study, the first dimension electrophoresis conditions differ only in temperature. The free energy changes obtained as a function of temperature therefore are related to the entrophy and enthalpy changes of the transition.

Three typical two-dimensional gels of pRW756 electrophoresed at 5, 18, and 35°C (in the first dimension) are shown in Fig. 2. We also conducted two-dimensional gels at 10, 22, and 28°C. Topoisomers labeled +1 to +4 (panel A), +1 to +6 (panel B), and +1 to +8 (panel C) represent positively supercoiled topoisomers. All the gels in Fig. 2 were run on an identical population of topoisomers. Therefore, the increase in the number of positive supercoils results from an increased unwinding of the DNA primary helix as the temperature is increased (34–36). The negative supercoils extend from the top center of the gel counterclockwise. In all cases, the topoisomers have a discontinuity at approximately $\tau = -14$ to $-15$. This discontinuity is indicative of Z-DNA formation and is related to a change in the writhe of the DNA. If the linking difference ($\alpha - \alpha_0$ or $\Delta Lk$) of the plasmid remains constant, according to the equation

$$\alpha - \alpha_0 = \Delta Lk = \Delta Tw + \Delta Wr$$

then the change in twist ($\Delta Tw$) and writhe ($\Delta Wr$) are related as $\Delta Tw = -\Delta Wr$. Thus, as the twist is changed by the formation of left-handed DNA, the writhe of the DNA is altered, and the mobility of the topoisomer is reduced. At 18 and 35°C, intermediates in the transition are observed as noted previously (10, 17, 19–21, 24, 27, 28). Furthermore, types of intermediates were found in the 5°C analysis which were not reported previously; the nature of all of these intermediates is poorly understood and is currently under further investigation.

Identical experiments to those performed on pRW756 were conducted on pRW155 at 5, 14, 22 and 36°C. The overall features of the two-dimensional gels obtained for pRW155 were similar to those obtained for pRW756. The major difference in the two sets of gels is that the first B to Z transition for pRW155 was observed at $\tau < -19$. The data obtained from this transition were used to calculate the free energy values for pRW155 as a function of temperature. Intermediates in the B to Z transition were also observed for pRW155 as reported previously for (dT–dG) plasmids (19, 27). Additionally, pRW155 has a second (dT–dG)$_6$ sequence that undergoes a B to Z transition at $\tau < -25$. Previously, it was shown that two (dC–dG) blocks in pRW751 of different lengths undergo the B to Z transition when sufficient supercoil pressure was applied (13, 18). In pRW751, however, the (dC–dG) blocks differed in length by 6 bp, whereas in pRW155 there are two identical length (dT–dG) blocks. The fact that there are two different transitions observed for pRW155 indicates therefore that one set of junction regions is more energetically favorable to the formation of left-handed DNA than the other set of junctions. This is the first demonstration that the energetics of the B to Z transition are influenced by the junction sequences.

**Calculation of $\Delta G_{BZ}$ and $\Delta G_{IO}$—Several different models for the thermodynamics of the B to Z transition in recombinant plasmids have been presented (24–26, 37). The gels in Fig. 2 effectively fit a statistical mechanical model which includes parameters for the free energy of the B to Z transition per bp, the free energy of junction formation, and the unwinding which occurs at the junction (24–26). The data from the two-dimensional gels were quantitated and fit to Equation 1 in the previous section by measuring the change in the helical twist ($\Delta Tw$) which occurs in topoisomers after the
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transition from B to Z DNA was initiated. The data pairs of linking difference \((a - a_b)\) and \((\Delta Tw)\) were subjected to a least squares fit which yielded values of \(\Delta G_{BZ}, \Delta G_{JX},\) and \(b\). A typical plot of \(-(a - a_b)\) versus \(- (\Delta Tw)\) obtained at 22 °C is shown in Fig. 3. The plot shows the power of using the linking number mutants of pRW756 (pRW58 and pRW59) to provide additional data points since small changes in plasmid size change the linking difference by less than a helical turn and hence the topoisormer mobility. The data points in Fig. 3 were fit to Equation 1 using a nonlinear least squares program which yielded the values of \(\Delta G_{BZ}\) and \(\Delta G_{JX}\), at temperatures of 5, 10, 18, 22, 28, and 35 °C. The \(\Delta G_{BZ}\) values ranged from 0.35 to 0.60 kcal/mol bp whereas the \(\Delta G_{JX}\) values ranged from 4.5 to 6.4 kcal/mol junction.

The data obtained from the two-dimensional gels of pRW155 were treated identically to the data obtained from pRW756. A typical plot of the linking difference \((a - a_b)\) versus the change in helical twist is shown with the theoretical fit in Fig. 4. It is important to note that the B to Z transition for pRW155, the (dT-dG)20-containing plasmid, occurs at a higher negative superhelical density than for the (dC-dG)20-containing plasmids (Fig. 3). This pattern was found at all temperatures studied (from 5 to 36 °C). The \(\Delta G_{BZ}\) values ranged from 0.78 to 0.80 kcal/mol bp whereas the \(\Delta G_{JX}\) values ranged from 4.4 to 4.9 kcal/mol junction.

Values of Entropy and Enthalpy Changes for B to Z Transitions—The free energy change for a given process is expressed as:

\[
\Delta G = \Delta H - T\Delta S
\]

which can be arranged to yield:

\[
\Delta G/T = \Delta H(1/T) - \Delta S
\]

We constructed plots (Fig. 5) of \(1/T\) versus \(\Delta G/T\) for \(\Delta G_{BZ}\) and \(\Delta G_{JX}\) values assuming that \(\Delta H\) and \(\Delta S\) remain constant over the temperature range studied for pRW756. The data were fit using a linear least squares program and the thermodynamic parameters for the B to Z transition (\(\Delta H_{BZ}, \Delta S_{BZ}, \Delta H_{JX},\) and \(\Delta S_{JX}\)) were calculated. The values of \(\Delta H\) were obtained from the slopes of the lines and the values of \(\Delta S\) were obtained from the negative of the intercepts. The most significant observation from this data on pRW756 (Table I) is that the \(\Delta H_{BZ}\) value is not near zero which is the value reported (3) for the salt-induced conformational transition for the DNA polymer (dG-dC)n.

\[
\Delta G_{BZ} = \Delta H_{BZ} - T\Delta S_{BZ}
\]

\[
\Delta G_{JX} = \Delta H_{JX} - T\Delta S_{JX}
\]
TABLE I

Thermodynamic parameters of B to Z transition in recombinant plasmids containing (dC-dG) in pR W756, pR W58, and pR W59 or (dT-dG) in pR W155

| Sequence       | B to Z | Junction |
|----------------|--------|----------|
|                | ΔH\(^\circ\) | ΔS\(^\circ\) | ΔH\(^\circ\) | ΔS\(^\circ\) |
| (dC-dG)        | -2.1   | -8.8     | 24      | 65       |
| (dT-dG)        | 0.58   | -0.76    | -1.8    | -2.2     |

\(\Delta H_{\text{BZ}}\) units, kcal/mol bp; \(\Delta S_{\text{BZ}}\) units, kcal/K-mol bp; \(\Delta H_{\text{BZ}}\) units, kcal/mol bp; \(\Delta S_{\text{BZ}}\) units, kcal/K-mol bp.

Fig. 6. Plots of 1/T versus ΔG/T for pR W155. Upper panel, data for a BZ base pair. \(\Delta H_{\text{BZ}}\) and \(\Delta S_{\text{BZ}}\) values are 0.58 kcal/mol bp and -0.76 kcal/K-mol bp, respectively. Lower panel, data for a BZ junction. The \(\Delta H_{\text{BZ}}\) and \(\Delta S_{\text{BZ}}\) values are -1.8 kcal/mol junction and -22 kcal/K-mol junction, respectively.

Similar analyses were performed on pR W155 (Fig. 6). These plots show that the \(\Delta H_{\text{BZ}}\) and \(\Delta S_{\text{BZ}}\) values for the B to Z transition in pR W155 are close to zero (Table I).

Theoretical Calculations from Thermodynamic Parameters—The contributions of the various thermodynamic factors to the B to Z transition were calculated from this data. Table I indicates that the two plasmids have differences in entropies and enthalpies depending on the base sequence. Temperature and chain length of the potential left-handed sequence also contribute to the thermodynamics of the B to Z transition in recombinant plasmids and therefore also influence the transition. Fig. 7 details the free energy contribution of the B to Z transition and the junctions. All the free energy contributions of supercoiling were omitted to provide a clearer illustration of the other factors involved in the transition. Panels A and B indicate that, as a function of temperature at a fixed chain length, the major thermodynamic factor which governs the B to Z transition in a plasmid containing a (dC-dG) sequence is the \(\Delta S_{\text{BZ}}\). In a plasmid containing a (dT-dG) sequence, however, the entropy change of the junction is the principal governing thermodynamic factor in the B to Z transition. This demonstrates that plasmids containing (dC-dG) sequences are more strongly influenced by the length of the block which can adopt a left-handed structure than plasmids containing (dT-dG) sequences. Panels C and D display the effect of the length of the potential left-handed sequence on the free energy. We have assumed that there is virtually no difference in the \(\Delta G_{\text{BZ}}\) with chain length as a consequence of studies by us and other workers (9, 18, 24, 27, 38). As the length of (dC-dG) sequence in a plasmid increases, the \(\Delta S_{\text{BZ}}\) governs the free energy change. In (dT-dG)-containing plasmids though, the \(\Delta H_{\text{BZ}}\) factor is the governing factor in the free energy change, that is, as the length of the (dT-dG) insert is increased, the \(\Delta H_{\text{BZ}}\) influences the free energy change more than the other factors.

Additionally, we evaluated directly the propensity of (dC-dG) and (dT-dG) sequences to adopt a left-handed structure as a function of temperature ignoring chain length and junction energy. We directly compared the \(\Delta H_{\text{BZ}}\) and \(\Delta S_{\text{BZ}}\) factors of (dC-dG) and (dT-dG) using Equation 3 and, surprisingly, found that the free energy changes for (dT-dG) sequences are more favorable than those for (dC-dG) sequences at temperatures above 60 °C.

DISCUSSION

This investigation of the thermodynamics of the B to Z transition shows that the ability of sequences to adopt left-handed conformations depends not only on sequence but also on temperature and length of the sequence. The B to Z transition in (dC-dG) plasmids is influenced by temperature much more than the B to Z transition in (dT-dG) plasmids. Additionally, longer sequences of (dT-dG) have transition free energies which are similar to the energies calculated for (dC-dG) plasmids. These different factors allow plasmids to undergo B to Z transitions under many different conditions. This flexibility which (dC-dG) and (dT-dG) sequences display may be utilized by cells to regulate biological processes. It is important, therefore, to evaluate these factors.

The thermodynamic factors \(\Delta H_{\text{BZ}}\) and \(\Delta S_{\text{BZ}}\) for (dC-dG) and (dT-dG) plasmids are substantially different. The \(\Delta H_{\text{BZ}}\) values for (dC-dG) plasmids are negative, whereas the \(\Delta H_{\text{BZ}}\) values for the (dT-dG) plasmid are positive. The \(\Delta S_{\text{BZ}}\) for the (dT-dG) plasmid, however, is substantially higher (an order of magnitude) than the \(\Delta S_{\text{BZ}}\) of (dC-dG) plasmids. This factor favors the formation of left-handed DNA in (dT-dG) plasmids at higher temperatures (above 60 °C). The observation of these transitions at higher temperatures is complicated, however, by the premelting and other structural features of plasmids which are observed at temperatures above 40 °C (36). The \(\Delta H_{\text{BZ}}\) and \(\Delta S_{\text{BZ}}\) values also influence the B to Z transition in (dC-dG) and (dT-dG) plasmids. The \(\Delta S_{\text{BZ}}\) contribution to the transition is considerably more favorable for (dC-dG) plasmids than for (dT-dG) plasmids. This factor may actually argue for a difference in the properties, and perhaps structure, of junctions involving (dT-dG) versus junctions involving (dC-dG). Although the thermodynamic data do not provide proof of a structural difference in the two types of junctions, if the structures were similar, we would expect similar values of \(\Delta H_{\text{BZ}}\) and \(\Delta S_{\text{BZ}}\). Two other pieces of evidence support this conclusion. First, the regions adjacent to (dC-dG) and (dT-dG) blocks in plasmids were shown to have different susceptibilities to OsO4 (39-41). Second, E. coli RNA
polymerase transcription is terminated by left-handed (dC-dG) blocks but not by left-handed (dT-dG) blocks (42). Our data suggest that the structure of the (dT-dG) junctions is more ordered based on a lower entropy change for (dT-dG) compared to (dC-dG) junctions. This difference in the junction structure may account for the inability of transcription to proceed through (dC-dG) blocks.

In addition to the differences based only on sequence, there are differences in the transition free energies which occur as a result of temperature changes. At temperatures above 60 °C, the values of $\Delta G_{BZ}$ for (dT-dG) plasmids are lower than for (dC-dG) plasmids. Thus, at higher temperatures the (dC-dG) sequences and (dT-dG) sequences form left-handed sequences interchangeably if the junction energy is not included.

The other factor which is critical to the formation of left-handed DNA in (dC-dG) and (dT-dG) plasmids is the length of the sequence of alternating pyrimidine-purine. Longer lengths of (dT-dG) above 60 bp form Z-DNA with a lower energy requirement than for (dC-dG) plasmids (at temperatures above 60 °C). Other workers (43) have postulated that at higher temperatures and longer chain lengths, (dC-dG) plasmids preferential form cruciform structures. The corresponding (dT-dG) plasmids, however, are not potential cruciform structures. It is also very interesting that there are many naturally occurring long stretches of (dT-dG), but relatively few base pairs of alternating (dC-dG) are found in natural sequences (44, 45). In fact, evidence suggests that in E. coli, there is a maximum length of (dC-dG) which is tolerated by the cells (31). This maximum length is at least partially dependent on the presence of recA-assisted repair (31).

In summary, we found that the thermodynamic factors for the B to Z transition in (dC-dG) and (dT-dG) plasmids are different. Additionally, both temperature and chain length affect the formation of left-handed DNA by changing the contributions to the free energy of the different thermodynamic factors. Furthermore, the thermodynamic factors associated with the BZ junctions are substantially different, which may be the result of different structural features in (dC-dG) junctions compared to (dT-dG) junctions. These features of our study predict that plasmids containing (dT-dG) sequences can undergo the B to Z transition with similar energies to the energies observed for (dC-dG) plasmids. Thus, this study has shown that there is a delicate interplay among sequence, temperature, and chain length factors that contribute to the overall free energy of the B to Z transition in recombinant plasmids.

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