Sequence-Dependent Effects of Spermine on the Thermodynamics of the B-DNA to Z-DNA Transition†

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Received July 29, 1996; Revised Manuscript Received October 3, 1996⊗

ABSTRACT: Spermine has been shown to bind to and stabilize a number of altered DNA conformations, including left-handed Z-DNA. Here, we have quantitatively studied the effects of spermine on the negative supercoil-induced transition from B- to Z-DNA. We have determined the intrinsic association constants for and the effective number of ligands that bind to both B- and Z-DNA. The intrinsic affinity of spermine for Z-DNA is ∼10 times higher for d(CA/TG) ($K_{ZP} = 1.2 \times 10^8$ M^{-1}) than for d(CG) dinucleotides ($K_{ZP} = 1.5 \times 10^7$ M^{-1}), and both are greater than that for B-DNA ($K_{BP} = 1.4 \times 10^5$ M^{-1}). This accounts for the stabilization of Z-DNA by spermine. The number of spermine accommodated by Z-DNA (n_Z) is sequence-dependent [$n_Z = 0.6$ spermine per 18 d(CA/TG) dinucleotides and 2.3 for 12 d(CG) dinucleotides]. The value of n_Z of <1 was interpreted as evidence for negative cooperativity in spermine binding to d(CA/TG) dinucleotides. Thus, although d(CA/TG) sequences saturate at lower spermine concentrations, the ligand has an overall greater effect on the stability of d(CG) dinucleotides as Z-DNA. B-DNA accommodates more spermines per base pair than either sequence as Z-DNA. At higher concentrations (>10 μM), spermine destabilizes Z-DNA. Using these parameters in a model for competitive spermine binding to B-DNA and Z-DNA, we can make predictions for how potential Z-DNA sequences found in the human genome are affected by cellular levels of superhelical density and spermine.

Although the molecular structure of left-handed Z-DNA was established over 15 years ago with the X-ray crystal structure of d(CGCGCG) (Wang *et al.*, 1979), the acceptance of Z-DNA as a biologically significant alternative to canonical right-handed B-DNA has been an area of constant debate. Many seem to feel that Z-DNA is formed only in crystals or under other "extreme" conditions such as very high salt. However, a growing body of work from the last several years suggests that all of the conditions required to induce Z-DNA formation do indeed exist inside cells. The cumulative results from these studies now provide a better appreciation for the fact that Z-DNA formation at discrete regions within the genomic DNA in the cell requires a dynamic combination of three major stabilizing factors: (i) relatively high levels of unconstrained negative supercoiling, (ii) the presence of Z-DNA-forming DNA sequences, and (iii) the presence of Z-DNA-stabilizing multivalent cations. Our current work focuses on the complex interplay of these factors on the stabilization of Z-DNA and provides some insight into the potential for Z-DNA as a biologically viable non-B-DNA structure.

When Liu and Wang (1987) first proposed the elegant "twin-domain" model of transcription-induced supercoiling, they predicted that the mechanism of transcription could theoretically generate levels of unconstrained negative supercoiling large enough to drive the formation of underwound structures, such as Z-DNA, behind a transcribing polymerase. Subsequent work from many labs has shown that transcription does lead to the dynamic generation of very high levels of both positive and negative supercoiling [reviewed in Freeman and Garrard (1992) and Droge (1994)]. Furthermore, the twin-domain model is general, since it applies to virtually any helix-tracking process, works both *in vivo* and *in vitro* and in both eukaryotes and prokaryotes. More importantly for this discussion, it has been shown that the process of transcription does indeed drive the formation of Z-DNA, as well as other non-B-DNA conformations inside cells. Rahmouni and Wells (1989) showed that Z-DNA-forming sequences placed upstream of an actively transcribing gene would induce formation of Z-DNA *in vivo*, but the same sequences placed downstream of the same transcribing gene would not form Z-DNA. Other work has shown that, in metabolically active nuclei, the binding of anti-Z-DNA antibodies to genomic DNA increases significantly during periods of active transcription (Wittig, *et al*. 1991). These results are consistent with many other experiments showing that, at least transiently, very high levels of negative supercoiling can exist inside cells as a direct result of the transcription process (Droge, 1994).

The second condition required for Z-DNA formation inside cells is the presence of potential Z-DNA-forming base sequences. Several early studies noted that alternating pyrimidine/purine (APP) sequences, such as poly-[d(CA)/d(TG)], are abundant in nature, especially in eukaryotic genomes (Hamada *et al.*, 1982a,b; Gross & Garrard, 1986). We had previously mapped the occurrence of Z-DNA in over 1 million bps of human DNA (Schroth *et al.*, 1992) and showed that potential Z-DNA-forming sequences of 12−16 bps are found about once every 3 kbp. More interestingly, these sequences were not randomly located in the genome. The strongest potential Z-DNA-forming sequences in humans are located more toward the 5′ end of genes and in promoter

† This work has been supported by grants from the National Science Foundation (MC B93 04467) and from the Environmental Health Science Center at Oregon State University (NIEHS ES00210). G.P.S. was supported by a Postdoctoral Fellowship from the American Cancer Society (PF-3749).

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⊗ Abstract published in *Advance ACS Abstracts,* November 15, 1996.

S0006-2960(96)01881-8 CCC: $12.00

Table 1: Sequences Used To Construct Plasmids pCG-24, pCA/TG-36, and pZ-40

pCG-24	d(agctCGCGCGCGCGCGCGCGCGCG)
pCA/TG-36	d(agctCACACACACACACACACACACACACACACACACA)
pZ-40	d(agctCCGCGCGCACGCGTGCGCACGCGTGCGCACGCGTGCGCGCGG)

[a] The four nucleotides of the *Hin*dIII restriction site are indicated by the lowercase letters, while those expected to form the B−Z junctions are underlined. The remainder of the sequence of the insert is expected to form standard Z-DNA. Each sequence forms a duplex having AGCT overhangs when properly annealed. The number associated with the names of each plasmid indicates the number of base pairs that should adopt the Z conformation. The number of dinucleotides in each insert that would form Z-DNA is thus half this value.

regions than near the 3′ ends of the gene. These results fit very well with the concept of the twin-domain model of transcription-induced supercoiling (Liu & Wang, 1987), as well as with the results of Rahmouni and Wells (1989). In other words, many of the strongest Z-DNA-forming sequences in the human genome are located at positions near 5′ ends of genes and therefore in regions which would be expected to have the maximum possible levels of negative supercoiling available from the transcription process.

The final stabilizing factor in the formation of Z-DNA is the effect of multivalent cations on the B-DNA to Z-DNA transition (the B−Z transition). Many cations such as magnesium, calcium, cobalt hexamine, and nickel have very strong stabilizing effects on the formation of Z-DNA in APP polymers such as poly-[d(CG)] [reviewed by Jovin *et al.* (1987) and Rich *et al.* (1984)]. Polyamines, which are biologically relevant multivalent cations, have been shown to be potent stabilizers of Z-DNA (Behe & Felsenfeld, 1981; Feuerstein *et al.*, 1992). Solution studies of the B−Z transition in both polymer DNA (Behe & Felsenfeld, 1981; Basu and Marton, 1987) and in supercoiled plasmids (Thomas *et al.*, 1991; Thomas & Thomas, 1994) have demonstrated that Z-DNA is dramatically stabilized by submillimolar concentrations of the naturally occurring polyamines spermidine and spermine. In this paper, we study the B−Z transition quantitatively using two-dimensional (2-D)[1] agarose gel electrophoresis in the presence of micromolar concentrations of spermine. These results allow us to study the effect that this polyamine has on the thermodynamics of Z-DNA formation and provide a basis for a model for quantitatively describing the ability of polyamines to stabilize Z-DNA. Using this model to analyze the binding data, we determined the binding constants of spermine for both Z-DNA and B-DNA, as well as the size of the spermine binding site for each form of DNA from our binding data. From this, we can make predictions for how intracellular levels of supercoiling and spermine concentrations affect the conformational behavior of Z-DNA-forming sequences.

MATERIALS AND METHODS

Z-DNA Plasmid Constructions. Alternating pyrimidine-purine sequences containing *Hin*dIII (5′-AGCT-3′) sticky ends (Table 1) were synthesized on an Applied Biosystems, Inc., 380B DNA synthesizer (Center for Gene Research and Biotechnology, Oregon State University), with the trityl-protecting group left on the 5′ end. The trityl-derivatized oligonucleotides were purified by NENsorb affinity chromatography (Dupont, Boston, MA) and subsequently desalted by size exclusion chromatography on a Sephadex G-10 column.

Plasmid DNAs were constructed by ligating the synthetic oligonucleotides directly into the *Hin*dIII restriction site of pBR322 (4263 bp in size). All plasmid DNAs were grown in *Escherichia coli* DH5α with chloramphenicol amplification and were isolated using Qiagen (Chatsworth, CA) purification kits followed by repeated phenol/chloroform extractions. The sequence of each plasmid insert was confirmed using an Applied Biosystems, Inc., 373A automated DNA sequencer (Center for Gene Research and Biotechnology, Oregon State University).

A broad distribution of topoisomers was prepared for the plasmids pCG-24, pCA/TG-36, and pZ-40 by adding various concentrations of ethidium bromide to each plasmid in the presence of topoisomerase I, as previously described (Peck *et al.*, 1982). Topoisomerase I was generously provided by Dr. Peter Yau of the University of California at Davis.

Two-Dimensional Gel Electrophoresis in the Presence of Spermine. The distributions of topoisomers were resolved by two-dimensional gel electrophoresis using a 1% agarose horizontal slab gel (20 cm × 20 cm) in a modified low-EDTA version of standard TBE buffer [90 mM Tris-borate and 0.15 mM EDTA (pH 8.3)] containing varying concentrations (0−30 μM) of spermine hydrochloride. The range of spermine concentrations used in these experiments was limited by the effect of the polyamine on the quality of the gels. In these experiments, spermine was added directly to the electrophoresis and sample buffers for the first dimension of electrophoresis. Low concentrations of spermine (0.1−5.0 μM) did not significantly affect the resolution of the agarose gel (Figure 1A,B). Higher concentrations of spermine (10−30 μM) (Figure 1C) caused some smearing in the first dimension, but the topoisomers could still be resolved in all cases. Precipitation and smearing of the topoisomer bands precluded the use of spermine at concentrations higher than 30 μM.

The topoisomers were pre-equilibrated in the modified TBE buffer containing spermine for 120 min at room temperature and then loaded in a well at the top left corner of the gel. The plasmids were separated according to their superhelical densities in the first dimension by electrophoresis at 90 V for 28 h. The gels were then equilibrated in a freshly prepared solution of TBE buffer containing 2.25 mg/mL chloroquine phosphate, but in the absence of spermine, for an additional 8 h and then electrophoresed in this same buffer for 24 h at 90 V. In all 2-D gel experiments, the reservoir buffer was recirculated continuously. The gels were stained with ethidium bromide and photographed with Polaroid 667 film. The gel images were digitized into a Macintosh IIcx computer using a HSD model SX100 scanner. The negative images of the gels were produced with Adobe Photoshop and used to index the topoisomers according to their degree of supercoiling (writhe, or ΔWr from migration along the first dimension), the inherent linking number (ΔLk from the second dimension), and helical twist [$\Delta Tw = \Delta Lk - \Delta Wr$ (Fuller, 1971)].

[1] Abbreviations: 2-D gel, two-dimensional gel electrophoresis; ΔLk, linking number relative to relaxed ccDNA; ΔWr, writhe or supercoiling relative to relaxed ccDNA; ΔTw, helical twist relative to relaxed ccDNA in the B form; *R*, gas constant (1.98 cal mol^{-1} K^{-1}); *T*, absolute temperature (kelvin); APP, alternating pyrimidine/purine; bp, base pair; ccDNA, closed circular DNA; dn, dinucleotide.

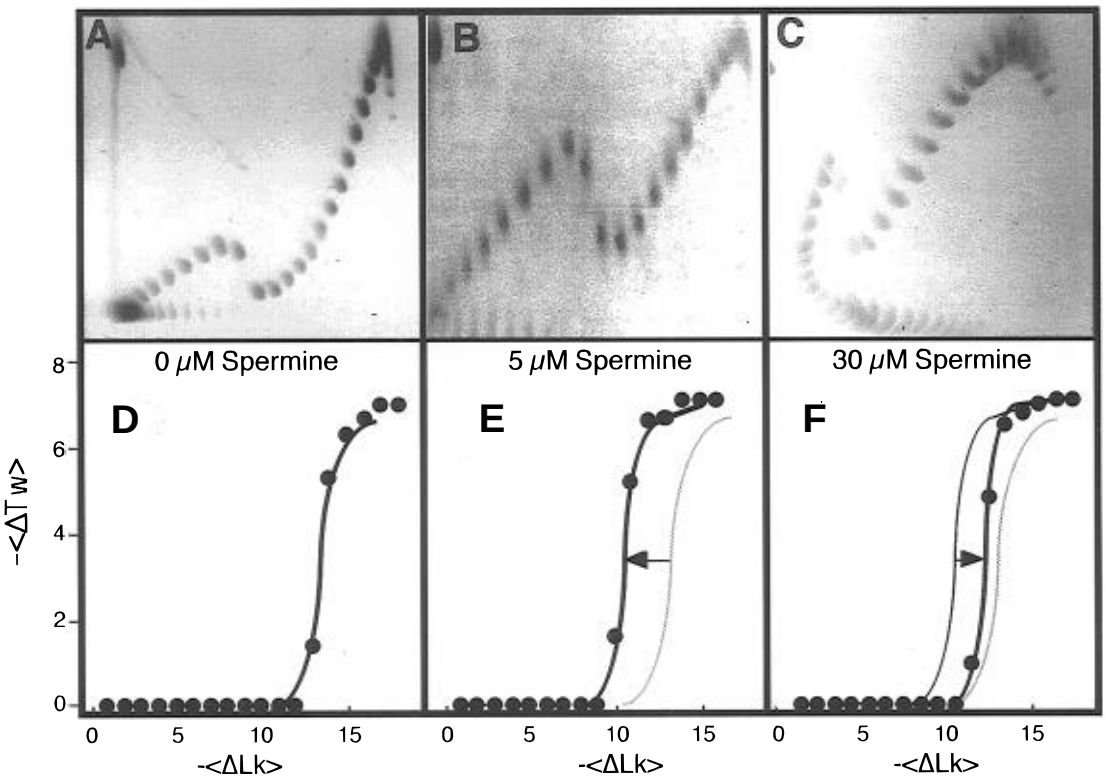

FIGURE 1: Effect of spermine on the B−Z transition of pZ-40 as measured by 2-D gel electrophoresis. (A−C) Topoisomer distributions of the plasmid pZ-40 were equilibrated with 0, 5, and 30 μM spermine hydrochloride and were resolved by 2-D gel in the presence of an equivalent concentration of spermine along the first dimension and in the absence of spermine but in the presence of 2.25 μg/mL chloroquine in the second dimension. The observed changes in helical twist ($\langle\Delta$Tw$\rangle$) were determined from the gels in panels A−C for each topoisomer having linking numbers ΔLk (results are plotted below each gel as $-\langle\Delta$Tw$\rangle$ versus $-\Delta$Lk). The data points were fit to a statistical mechanics treatment of the zipper model that describes the B−Z transition (described in the text). The solid curves represent the best fit to each data set (closed circles). The dashed curves in plots B and C represent the B−Z transition in 0 μM spermine (plot A). The light solid curve in plot C represents the fitted curve for the B−Z transition in 5 μM spermine. Arrows indicate the direction of the shift in ΔLk for the midpoint of the transition going from 0 to 5 μM (plot B) and from 5 μM to 30 μM spermine (plot C).

Determining B−Z Transition Free Energies from 2-D Gels. The observed free energy of the B−Z transition ($\Delta G_{obs}°$) was determined by fitting the ΔTw observed from the 2-D gel analyses to ΔLk of each topoisomer using a statistical mechanics treatment of the zipper model (Peck & Wang, 1983; Vologodskii & Frank-Kamenetskii, 1984; Ellison *et al*., 1985; Mirkin, 1987; Ho 1994). In this treatment, we first define the partition function (Q) for all possible states in terms of the equilibrium constants and unwinding of the ccDNA for the nucleation step (σ_N and b) and the equilibrium constant and unwinding for propagating Z-DNA through each jth dn (S_j and a_j) in a sequence of n dinucleotides within a plasmid of N bp (eq 1). Here, $K = 1100RT/N$, where N is the size of the plasmid pBR322 (4263 bp) plus the length of the plasmid insert (Table 1) and ΔLk is the linking number for a given topoisomer.

$$Q = 1 + \sum_{i=1}^{n}\sum_{k=1}^{n}\sigma_N\left(\prod_{j=i}^{k}S_j\right)\exp\left[\frac{-K}{RT}\left(\Delta\mathrm{Lk} - \sum_{j=i}^{k}a_j - 2b\right)^2\right] \quad (1)$$

The average unwinding ($\langle\Delta$Tw$\rangle$) due to the B−Z transition is calculated as the probability function in eq 2.

$$\langle\Delta\mathrm{Tw}\rangle = Q^{-1}\left[\sum_{i=1}^{n}\sum_{k=1}^{n}\left(\sum_{j=1}^{k}a_j + b\right)\sigma_N\left(\prod_{j=i}^{k}S_j\right)\times \exp\left[\frac{-K}{RT}\left(\Delta\mathrm{Lk} - \sum_{j=i}^{k}a_j - 2b\right)^2\right]\right] \quad (2)$$

The values for $\Delta G_{obs}°$ (defined as the sum of the nucleation and propagation free energies) were determined by treating the free energy for nucleation ($\Delta G_N° = -RT\ \ln\sigma_N$) and propagation [$\Delta G_P° = -RT\ \ln(\Pi S_j)$] of Z-DNA as variables and systematically varying each term to converge at a minimum value for the sum of squares difference between the calculated and observed $\langle\Delta$Tw$\rangle$ for each topoisomer. Calculations were performed using the program 2DANAL on a MicroVax II GPX workstation (Digital Equipment, Corp.). The errors in determining $\Delta G_{obs}°$ were defined primarily by our ability to accurately assign ΔLk and ΔTw from the 2-D gels and not by the error in the fitting of the parameters to the statistical mechanics model. For conditions where the gels were run in triplicate, we found this to be $\pm$ 0.25 turn, which translates to errors in $\Delta G_{obs}°$ ranging from $\pm$0.5 to $\pm$0.8 kcal/mol.

Although $\Delta G_{obs}°$ is determined as the sum of $\Delta G_N°$and $\Delta G_P°$, the overall free energy is not generally biased by the model. An underestimate of, for example, $\Delta G_N°$ would be compensated for by an increase in $\Delta G_P°$ such that the total $\Delta G_{obs}°$ remains constant. Thus, the partitioning of energy between $\Delta G_N°$ and $\Delta G_P°$ affects primarily the cooperativity of the simulated transition and not the midpoint where $\Delta G_{obs}°$ is defined. This analysis yields values for $\Delta G_{obs}°$ that are very similar to those from methods that require interpolation of the curves to the midpoint of the transition. The fit to the statistical mechanics model, however, is more accurate since it does not require a determination for the end point of the transition (as required to accurately determine a

Table 2: Effect of Spermine on the Observed B−Z Transition Free Energy ($\Delta G_{obs}°$) for Each Sequence in Table 1[a]

	$\Delta G_{obs}°$		
[spermine] (μM)	pCG-24	pCA/TG-36	pZ-40
0.0	15.0	29.8	24.5
0.1	ND[b]	28.7	ND
0.5	12.0	28.0	19.7
2.0	10.9	27.7	17.6
5.0	8.6	27.9	15.7
10.0	9.4	28.3	15.8
15.0	9.5	28.5	17.7
20.0	9.4	ND	17.9
30.0	9.6	ND	19.4

[a] The values for $\Delta G_{obs}°$ (in kilocalories per mole) were determined as described in the text. [b] ND, not determined.

midpoint), and it uses all the data in and around the midpoint to determine $\Delta G_{obs}°$.

Once values for $\Delta G_{obs}°$ were determined for each sequence at different spermine concentratrions (Table 2), the data were analyzed with various equilibrium binding models by fitting the parameters of the models using a nonlinear least-squares routine (in the program Scientist 2.0 for Windows, MicroMath Scientific Software, Inc., Salt Lake City).

RESULTS

Monitoring the B−Z Transition by Two-Dimensional (2-D) Gel Electrophoresis. We have assessed the effect of DNA binding ligands on the stability of Z-DNA by monitoring the changes in topological properties [the linking number (ΔLk), helical twist (ΔTw), and writhe (ΔWr)] of ccDNA. The basic experimental design for measuring these parameters by 2-D gel and a statistical mechanics approach to determine the topological effects of a B−Z transition have been previously described (Peck & Wang, 1983; Vologodskii & Frank-Kamenetskii, 1984; Ellison *et al.*, 1985; Mirkin *et al*., 1987; Ho, 1994). In short, we follow the formation of Z-DNA in sequences inserted into pBR322 (Table 1) by resolving topoisomers of the plasmid using 2-D gel (Figure 1A−C). The B−Z transition free energies ($\Delta G_{obs}°$) are determined from the 2-D gels by fitting the observed average $\langle\Delta Tw\rangle$ at each topoisomer using a statistical mechanics treatment of the zipper model for the B−Z transition (Peck & Wang, 1983; Vologodskii & Frank-Kamenetskii, 1984; Ellison *et al.*, 1985; Mirkin, 1987) (Figure 1D−F).

Effectors such as spermine that stabilize Z-DNA by differential binding to the left-handed Z and the right-handed B conformations can be studied by including various concentrations of the ligand in the buffer for the first dimension of the 2-D gel. The ligand is absent in the second dimension so that the B−Z transition is readily reversed. In the experiments presented here, 0.1−30 μM spermine was included in the buffers used to cast the gels and in the buffers for the first dimension of electrophoresis. Since the volumes of the buffers containing spermine are extremely large as compared to that of DNA, the polyamines are not readily depleted and thus represent the "free" ligand concentrations ([P]). This greatly simplifies the subsequent quantitative analysis of the results. The studies are additionally simplified in that the 2-D gels monitor only the behavior of the sequences that have been inserted into the plasmid. The remainder of the ccDNA does bind spermine, but the large reservoir of spermine available from the buffer suggests that we do not need to consider the competition for ligand binding at the sequence being monitored versus the remainder of the ccDNA.

Spermine binding to the overall plasmid may affect the relationship between free energy and supercoiling. The overall distribution of topoisomers, however, was observed to remain unchanged (ΔLk = +5 to −35 turns) over the spermine concentrations used in these studies (Figure 1A−C), indicating that the relationship between the free energy and levels of supercoiling in the plasmids had not been significantly perturbed. If there were a ligand effect, we would expect to observe a shift to more positive (less negative) supercoiled topoisomers if spermine favored positive supercoiling, to more negative (less positive) supercoiled topoisomers if spermine favored negative supercoiling, or to less of both types if spermine disfavored either form by making the DNA stiffer. Since this was not observed, we did not consider the overall behavior of the ccDNA to be perturbed by the presence of spermine. Thus, in these studies, the values determined for $\Delta G_{obs}°$ reflect the effect of spermine binding on the relative stability of each sequence listed in Table 1 as B- or Z-DNA.

Effect of Spermine on the B−Z Transition Free Energies. In the current work, we studied the effect of spermine on the B−Z transition for three different Z-forming sequences (Table 1). These allow us to determine whether spermine affects Z-DNA differently for d(CG), d(CA/TG), or combinations of the two types of dinucleotides. Throughout this paper, we will discuss all Z-DNA-forming sequences as combinations of dinucleotides, since the dinucleotide (dn) is the fundamental repeating structural unit for Z-DNA (Rich *et al.*, 1984). In these studies, adding low concentrations of spermine (0 to 5−10 μM) to the running buffer of the 2-D gels effectively reduces the number of negative supercoils (and the associated free energy) at which the B−Z transition is observed (Figure 2) for these sequences. At higher spermine concentrations, however, the transition shifts back to higher superhelical densities (more negative ΔLk). These effects are reflected in the decrease in the $\Delta G_{obs}°$ values in all three sequences studied at low ligand concentrations (Table 2). At higher concentrations, however, $\Delta G_{obs}°$ increases in all cases. This is similar qualitatively to the behavior reported by Thomas *et al.* (1991), but here, we can accurately quantify the effect of the ligand on $\Delta G_{obs}°$ for the B−Z transition. The $\langle\Delta Tw\rangle$ associated with the transitions in all cases did not change with spermine concentration, but remained at values expected for differences between B- and Z-DNA for each sequence. Thus, throughout the spermine range, the assay monitored the B−Z transition, even though there are apparently two competing effects of ligand on the transition.

To gain a better understanding for the Z-DNA-stabilizing effect at low polyamine concentrations and the destabilizing effect at high polyamine concentrations, we can further analyze $\Delta G_{obs}°$ by considering models for ligand interactions with DNA. The opposing behavior of $\Delta G_{obs}°$ at low and high spermine concentrations suggests that the simplest treatment of the titrations requires two competing binding equilibria.

Model for Spermine Binding to Plasmid Inserts. Our working model to describe the effect of spermine on the stability of Z-DNA is shown in Scheme 1.

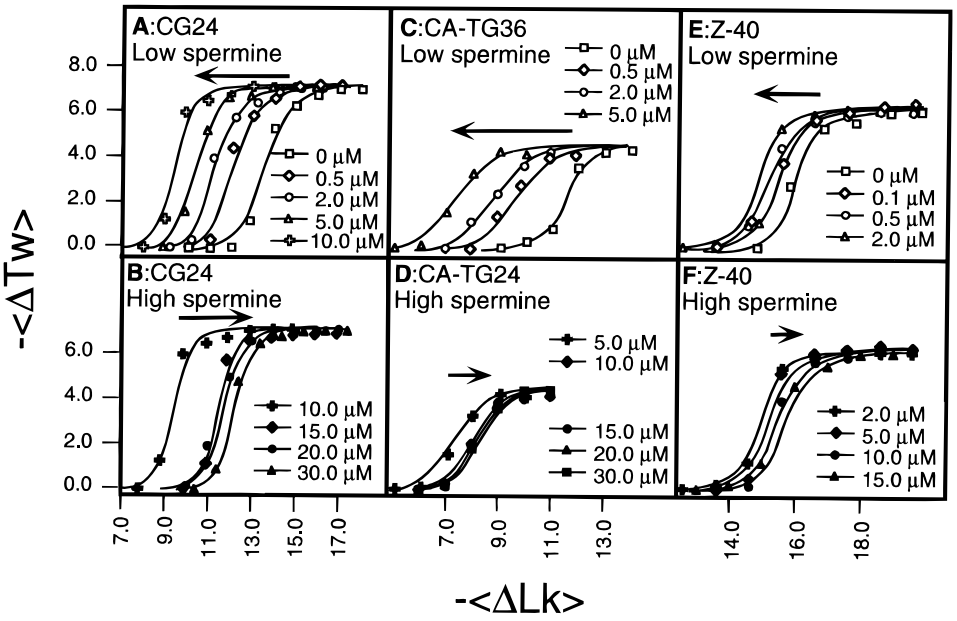

FIGURE 2: Effect of spermine on the B−Z transition observed in pCG-24 [(A) at low spermine and (B) at high spermine concentrations], pCA/TG-36 [(C) in low spermine and (D) in high spermine concentrations], and pZ-40 [(E) in low spermine and (F) in high spermine concentrations]. Curves were fit using the statistical mechanics treatment of the zipper model that describes the B−Z transition (eqs 1 and 2 in the text). Arrows indicate the direction in which the midpoints of the transitions shift as the spermine concentration is increased.

Scheme 1

$$BP_n \underset{n_B}{\overset{K_{BP}}{\rightleftharpoons}} B \overset{K_{B-Z}}{\rightleftharpoons} Z \overset{n_Z,\ K_{ZP}}{\rightleftharpoons} ZP_n$$

Here, the plasmid insert is in equilibrium between B- and Z-DNA (K_{B-Z}). Spermine binds competitively to either form. The parameters K_{BP} and K_{ZP} represent the association constants and n_B and n_Z the number of sites for polyamine binding to B- and Z-DNA, respectively. The observation that low spermine concentrations reduce $\Delta G_{obs}°$ indicates that spermine has a higher affinity for Z-DNA than for B-DNA ($K_{ZP} > K_{BP}$) in all the sequences studied. Using this model to analyze $\Delta G_{obs}°$, we will show that the number of spermine binding to B-DNA must be greater than for the Z form in order for the free energy to increase again at high spermine concentrations.

Starting with this model, we can derive a simple relationship between $\Delta G_{obs}°$ and the concentration of free polyamine ([P]) present in the gels. At any given concentration of spermine, the overall equilibrium associated with the B−Z transition (K_{obs}) is the sum of all the the unbound (Z_u) and bound forms of Z-DNA ($\sum Z_b$) and all the unbound (B_u) and bound forms of B-DNA ($\sum B_b$) for the plasmid inserts (eq 3).

$$K_{obs} = \frac{Z_u + \sum Z_b}{B_u + \sum B_b} = \frac{Z_u/B_u + \sum Z_b/B_u}{1 + \sum B_b/B_u} \quad (3)$$

By relating this to the equilibrum constants in Scheme 1, eq 3 becomes eq 4.

$$K_{obs} = \frac{Z_u/B_u + (\sum Z_b/Z_u)(Z_u/B_u)}{1 + \sum B_b/B_u} = \frac{K_{B-Z}\,[1 + (\sum Z_b/B_u)]}{1 + \sum B_b/B_u} \quad (4)$$

Finally, this can be related to $\Delta G_{obs}°$ and $\Delta G_{B-Z}°$ (the B−Z transition free energy in the absence of spermine) by eq 5.

$$\frac{K_{obs}}{K_{B-Z}} = \frac{1 + \sum Z_b/Z_u}{1 + \sum B_b/B_u} \text{ or } \Delta G_{obs}° - \Delta G_{B-Z}° = -RT[\ln(1 + \sum Z_b/Z_u) - \ln(1 + \sum B_b/B_u)] \quad (5)$$

Equation 5 is a general expression for the competitive model in Scheme 1. To use this to determine the intrinsic spermine binding constants for B- and Z-DNA (K_{BP} and K_{ZP}) and the number of ligands bound per insert (n_B and n_Z, respectively), the ratios $\sum Z_b/Z_u$ and $\sum B_b/B_u$ need to be defined in terms of a formalism for analyzing binding data. Braunlin *et al.* (1982) showed that results from equilibrium dialysis studies on spermine binding could be fit by a Scatchard analysis as well as by the more complete analysis for ligand binding to DNA as derived by McGhee and von Hipple (1974). We initially adapt the Scatchard analysis to analyze the spermine binding data. By defining $\sum Z_b$ as the total Z forms of an insert minus Z_u and similarly $\sum B_b$ as the total B-DNA minus B_u, we can readily derive an expression (eq 6) to introduce the parameters K_{BP}, $K_{ZP,}$, n_B, and n_Z that takes into account the statistical weights for all possible bound forms of the insert, assuming all the binding sites are identical and independent.

$$\Delta G_{obs}° - \Delta G_{B-Z}° = -RT[n_Z \ln(1 + K_{ZP}[P]) - n_B \ln(1 + K_{BP}[P])] \quad (6)$$

Using a nonlinear least-squares routine to fit the parameters of this expression to the data in Table 2, however, yielded results that were, unfortunately, uninterpretable (not shown). The values for n_B were at least 4 times higher than previously reported (Braunlin *et al.*, 1982), and none of the binding constants was significant in terms of their estimated standard errors. For example, K_{ZP} was estimated to be $\sim 10^{12}$ M^{-1}, while the standard error was on the order of 10^{13} M^{-1}. Our attempts to apply the McGhee and von Hipple (1974) analyses for noninteracting sites gave similar results. It was apparent that the standard formalisms for ligand binding to linear DNA were not appropriate for our binding data.

In hindsight, the likely reason these analyses did not work is because they treat the DNA as an infinite polymer with identical binding sites. The plasmid inserts that we monitor in these studies are flanked at both ends by junctions between right-handed B-DNA and left-handed Z-DNA (the B−Z junctions). Thus, the DNA that converts to Z-DNA is not infinite but has well-defined ends (Peck & Wang, 1983). In addition, the inserts are not uniformly Z-DNA. Although the B−Z transition is cooperative, and is often treated as an all-or-none process, both simulations using the zipper model (Ho, 1994) and chemical (Johnston *et al.*, 1988; Kladde *et al.*, 1994) and nuclease assays (Hayes & Dixon, 1985) indicate that Z-DNA induced by negative supercoiling, particularly in d(CA/TG)$_n$ sequences, is nonuniformly propagated through the inserts. Thus, the binding sites in the plasmid inserts are not equivalent across the sequence. We could at this point attempt to apply the more general form of the McGhee and von Hipple (1974) model for cooperative and nonequivalent binding sites to analyze the data, but this would have introduced more parameters and complexity than supported by the quantity or the quality of the data. Instead, we decided to derive a simpler model in which all the bound forms of the Z insert ($\sum Z_b$) are treated as a single average species with an effective binding constant K_{ZP} and an average effective number of binding sites n_Z. The $\sum B_b$ species was similarly treated to define an effective binding constant K_{BP} and an average effective number of binding sites n_B. Obviously, this is not as rigorous as the McGhee and von Hippel (1974) treatment of ligand binding to DNA, but it greatly reduces the complexity and the number of parameters required to fit the model.

By defining the bound forms of the insert as single average species, we do not define statistical weights. Thus, $\sum Z_b/Z_u = (K_{ZP}[P])^{n_Z}$ and $\sum B_b/B_u = (K_{BP}[P])^{n_B}$. Substituting these into eq 5, we now have a simple expression to describe spermine binding to the plasmid inserts as B- and Z-DNA (eq 7)

$$\Delta G_{obs}^\circ - \Delta G_{B-Z}^\circ = -RT[\ln[1 + (K_{ZP}[P])^{n_Z}] - \ln[1 + (K_{BP}[P])^{n_B}]] \quad (7)$$

In using eq 7 to analyze the effect of spermine on the free energy observed for the B−Z transition, we can determine the intrinsic equilibrium binding constants of the polyamine to both right-handed B-DNA and left-handed Z-DNA and the effective number of ligands bound to the plasmid inserts.

We started by fitting the parameters of eq 7 to the data in Table 2 using a nonlinear least-squares routine. Values obtained for the B-DNA parameters K_{BP}, and n_B were significant and consistent across all three sequences (Table 3). The values for the Z form, although more reasonable than those obtained from the Scatchard or the McGhee and von Hipple analyses, showed high standard errors. Thus, although the number of parameters for the model was reduced to only four, the confidence in the fitted parameters (particularly the K_{ZP}) was low because of the small number of data points for each insert. We attempted to further reduce the number of parameters by first defining an average K_{BP}, and n_B (normalized and redefined as the size of the spermine binding site, s_B) for B-DNA. These parameters are independent of base composition (Hirshmann *et al.*, 1967) and, by inference, of sequence. These average B-DNA parameters were then used to determine K_{ZP} and n_Z for each insert as Z-DNA.

Table 3: Thermodynamic Parameters Determined for Spermine Binding to B-DNA[a]

plasmid	n_B (spermines/insert)	$K_{BP} \times 10^{-5}$ (M^{-1})	s_B (bp/spermine)
pCG-24	3.4 (1.0)	1.6 (1.1)	7.1 (2.2)
pCA/TG-36	5.6 (1.5)	1.2 (0.2)	6.4 (1.4)
pZ-40	8.1 (0.7)	1.3 (0.1)	4.9 (0.5)
average		1.4 (0.2)	6.1 (1.1)

[a] The number of spermines (n_B) and the association constant for spermine binding (K_{BP}) to each sequence of plasmids pCG-24, pCA/TG-36, and pZ-40 (Table 1) were determined as described in the text. The size of the spermine binding site (s_B) was calculated as N/n_B, where N is the size of the APP sequence that adopts the Z conformation. [b] The standard deviations for all values are shown in parentheses.

Binding of Spermine to Inserts as B-DNA. The parameters for spermine binding to the inserts in their B form determined here (Table 3) were consistent between the sequences and with previously published data. The value for K_{BP} (average $= 1.4 \times 10^5$ M^{-1} spermine) is identical for all the sequences in this study and is comparable to that obtained from more conventional methods. The association constant of spermine to B-DNA was reported by Braunlin *et al.* (1982) to be salt-dependent (ranging from $\sim 10^3$ to $\sim 10^4$ M^{-1}, for [Na^+] from 0.154 to 0.071 M). A 2-fold decrease in [Na^+] was shown to increase the association constant by a factor of 10. In our studies, the spermine binding constants were determined in a buffer system containing no added Na^+ and ~0.15 mM EDTA. This system therefore can be considered to be essentially depleted of competing cations. It is not surprising, therefore, that the intrinsic binding constant determined here is approximately 10 times greater than that previously published. We can estimate from K_{BP} and the relationships of Braunlin, *et al.* (1982) that [Na^+] $\approx$ 0.036 M in our studies.

The number of ligands bound to each sequence, when normalized for length differences, was on average 0.17 spermine/bp. This translates to an average value for s_B of 6.1 ± 1.1 bp/spermine. This is higher than the 4.8 bp/spermine reported (Braunlin *et al.*, 1982); however, again, the equilibrium dialysis studies showed that s_B for the polyamines spermine, spermidine, and most strongly for putrescine was generally higher at lower sodium salt concentrations, although an exact relationship was not determined. Thus, under the lower salt conditions of the current studies, it is not surprising that s_B is slightly higher than previously reported.

Binding of Spermine to Inserts as Z-DNA. With the average values for B-DNA defined, K_{BP} and s_B were fixed and used in a nonlinear least-squares fit of eq 7 to determine K_{ZP} and n_Z (Table 4). K_{ZP} for pCA/TG-36 is 10 times greater than that for pCG-24, indicating the preference of the polyamine for the left-handed form of d(CA/TG) over d(CG) dinucleotides. This is reflected in the lower concentrations of spermine required to saturate the pCA/TG-36 sequence as opposed to the pCG-24 sequence. The effective number of spermines that bind to pCG-24 ($n_Z = 2.3$ molecules) was, however, observed to be greater than that of pCA/TG-36 ($n_Z = 0.6$ molecule), even though the latter sequence was longer. Thus, at saturation, the effect of spermine on ΔG_{obs}° is greater for pCG-24 (~6.2 kcal/mol of stabilization) than for pCA/TG-36 (only ~2.2 kcal/mol of stabilization).

Table 4: Thermodynamic Parameters Determined for Spermine Binding to Z-DNA[a]

plasmid	n_Z (spermines/insert)	$K_{ZP} \times 10^{-7}$ (M^{-1})	s_Z (bp/spermine)
pCG-24	2.3 (0.1)	1.5 (0.5)	10.4 (0.5)
pCA/TG-36	0.66 (0.05)	12 (7)	55 (4)
pZ-40	2.5 (0.1)	4.9 (1.4)	16.0 (0.5)

[a] The number of spermines (n_Z) and the association constant for spermine binding (K_{ZP}) to the Z-DNA conformation of the plasmids pCG-24, pCA/TG-36, and pZ-40 (Table 1) were determined from eq 7 in the text, with K_{BP} and n_B fixed. The size of the spermine binding site to Z-DNA (s_Z) was calculated as N/n_Z, where N is the number of base pairs in each Z-DNA forming sequence. [b] The standard deviations for all values are shown in parentheses.

For all sequences, the affinity of spermine for Z-DNA is sequence-dependent and is at least 2 orders of magnitude greater than for B-DNA. Thus, it is not surprising that spermine stabilizes Z-DNA. What surprised us was that, at a [P] of $\geq 10-15$ μM, $\Delta G_{obs}°$ increases. The magnitude of this increase was dependent not on the difference in the equilibrium constants for the sequences but on the effective number of spermines that bind to the B versus Z form of each sequence. The B form of pCG-24 accommodates one additional spermine molecule as compared to the Z form. This is a small difference between the two conformations, and subsequently, the degree of destabilization of Z-DNA was not very large ($\sim$1 kcal/mol for [P] = 30 μM). For pCA/TG-36, the B form accommodates $\sim$10 times the number of spermine that the Z form does. Thus, at high spermine concentrations, $\Delta G_{obs}°$ approaches the initial value (with no added spermine). Indeed, this latter sequence in 20 μM spermine is predicted to be less stable as Z-DNA by $\sim$0.5 kcal/mol than in the absence of spermine and by 30 μM would become nearly 2 kcal/mol less stable.

The sequence pZ-40 is a combination of 14 d(CG) and 6 d(CA/TG) dinucleotides. Not surprisingly, the effect of spermine on this sequence is intermediate between that on pCG-24 and pCA/TG-36. The effective number of spermines bound to the Z form of this sequence was observed to be proportional to its composition. Using the values of n_Z from pCG-24 and pCA/TG-36, we can estimate that the 14 d(CG) dinucleotides would bind $\sim$2.6 spermines with a K_{ZP} of 1.5×10^7 M^{-1}, that the 6 d(CA/TG) dinucleotides would bind 0.22 spermine with an affinity constant of 1.2×10^8 M^{-1}, or that the average value across the entire sequence is predicted to be $n_Z = 2.9$ spermines and $K_{ZP} = 2.8 \times 10^7$ M^{-1}. This assumes that all dinucleotides contribute equally to these parameters. The observed values of $n_Z = 2.6$ spermines and $K_{ZP} = 4.9 \times 10^7$ M^{-1} are, within the error of the estimation, in the range of the predicted values. Thus, the behavior of this mixed sequence is dominated by the d(CG) dinucleotides because of the larger number of spermine molecules that bind to this type of dinucleotide.

We have determined the association constants (K_{BP} and K_{ZP}), the effective number of ligands (n_B and n_Z), and the associate size of the spermine binding site (s_B and s_Z) for various sequences as B- and Z-DNA. The final form of the model used to analyze the data is admittedly less rigorous than that presented by McGhee and von Hippel (1974) to describe ligand binding to linear polymers such as DNA. We do not explicitly treat the cooperativity or the possible overlap of binding sites. The effects of cooperativity are likely to be reflected in n_B and n_Z (Braunlin *et al.*, 1982). The value that we obtained for s_B, however, seems to be reasonable compared to that previously reported (Braunlin *et al.*, 1982). More importantly, the model and the parameters derived here for spermine binding to B and Z forms of the plasmid inserts very accurately simulate the effects competitive binding of spermine has on the free energy of the B−Z transition (Figure 3). Thus, the general conclusions that can be drawn from these studies are valid and insightful for understanding and predicting how spermine affects the negative supercoil-induced transition from B- to Z-DNA.

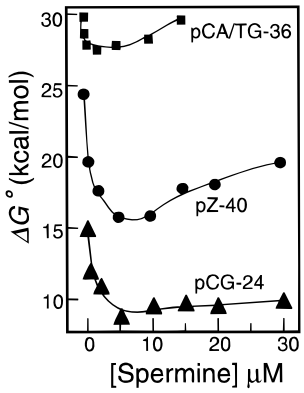

FIGURE 3: Comparison of the effects of increasing spermine concentrations on the B−Z transition free energies ($\Delta G°$) as observed (data points) and simulated (curves) for pCG-24 (triangles), pCA/TG-36 (squares), and pZ-40 (circles). The curves were calculated using eq 7 in the text, with parameters for B-DNA in Table 3 and parameters for Z-DNA in Table 4.

DISCUSSION

We have observed that spermine reduces the superhelical density at which the B−Z transition is observed in a 2-D gel assay. Thus, Z-DNA is stabilized by the polyamine, as previously observed in polymers (Behe & Felsenfeld, 1981; Feuerstein *et al.*, 1992) and in supercoiled plasmids as assayed by anti-Z-DNA antibodies (Thomas *et al.*, 1991; Thomas & Thomas, 1994). The stabilization of Z-DNA by spermine does not increase monotonously as the ligand concentration increases but is reversed at concentrations greater than $\sim$10 μM spermine. This effect had previously been reported for short d(CG)$_n$ sequences inserted into ccDNA (Thomas *et al.*, 1991), with speculation that perhaps a transition to another undefined conformation was induced at high spermine concentrations. We show here that this destabilization is inherent in the competitive binding of spermine to B- to Z-DNA.

The model presented in Scheme 1 and parametrized in eq 7 provides a quantitative explaination for both the stabilizing and destabilizing effects of spermine by defining values for the affinity constants and the effective number of ligand sites for spermine binding to different sequences as B- and Z-DNA. For B-DNA, these parameters were sequence-independent and are similar to previously reported values (Braunlin *et al.*, 1982). The parameters for Z-DNA, in contrast, were sequence-dependent.

The association constant for d(CG) dinucleotides is 100-fold greater for Z-DNA than for B-DNA. The size of the binding site for sequences of d(CG)$_n$ is $\sim$10 bp/spermine or

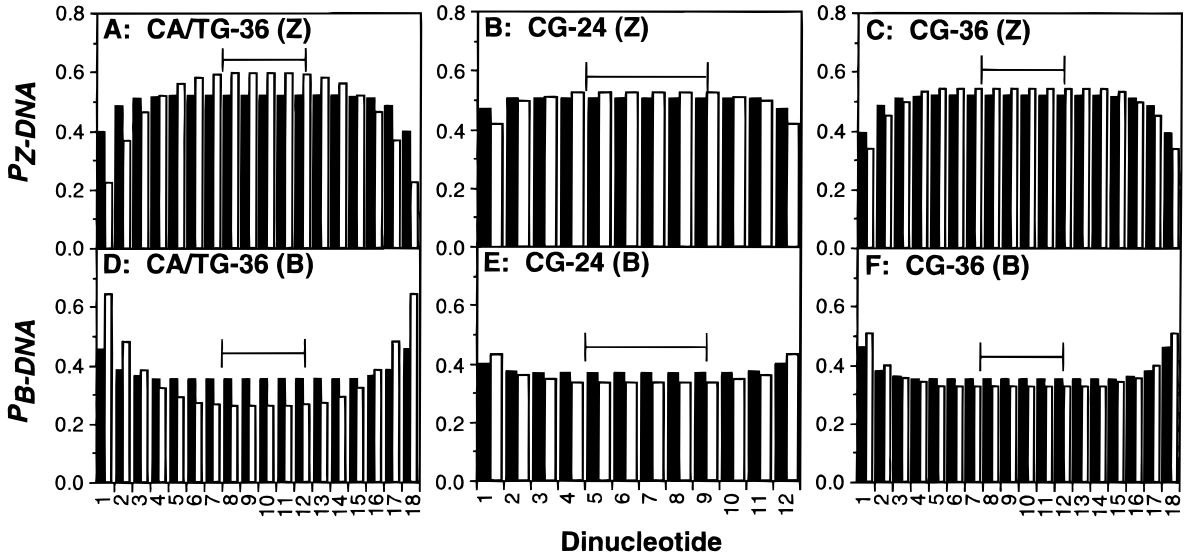

FIGURE 4: Effect of spermine binding on the distribution of Z-DNA and B-DNA. The probabilities that any dinucleotide in a sequence of 18 d(CA/TG) dinucleotides will be Z-DNA (A) or B-DNA (B), of 12 d(CG) dinucleotides will be Z-DNA (C) or B-DNA (D), and of 18 d(CG) dinucleotides will be Z-DNA (E) or B-DNA (F) were calculated using eq 7 in the text. The solid columns represent the probabilities for Z-DNA (P_{Z-DNA}) or B-DNA (P_{B-DNA}) in the absence of spermine, while the open columns are in the presence of spermine. The horizontal bars indicate the dinucleotides that were defined as bound by spermine in this simulation. The effect of binding one spermine to ~10 bp (five dinucleotides) within each sequence was modeled by reducing the $\Delta G_P°$ by a value equal to $-RT \ln K_{ZP}$ for each dinucleotide type (Table 4). For the d(CA/TG) sequence, $\Delta G_P°$ for each bound dinucleotide was assigned to be −0.8 kcal/mol, which is 2.2 kcal/mol lower than the 1.4 kcal/mol value for the unbound dinucleotides. Each bound d(CG) dinucleotide was assigned a $\Delta G_P°$ of −1.4 kcal/mol, which is 2 kcal/mol lower than the 0.6 kcal/mol defined for the unbound dinucleotides.

about 1 spermine molecule per turn of Z-DNA. This however differs from the 2 ligands observed per turn of Z-DNA for the intramolecular spermine in the −100 °C crystal structure of $d(CG)_3$. The crystal structure suggests that $s_Z \approx 6$ bp/spermine, as opposed to 10 bp/spermine observed here. This discrepancy may be related to the crystallographic constraint of having half a turn of Z-DNA as the asymmetric unit in the crystal.

A d(CA/TG) dinucleotide as Z-DNA has ~10-fold higher affinity for spermine as compared to d(CG). This is consistent with what has been previously reported (Thomas & Thomas, 1994). How do we explain, however, the small number of ligands that effectively bind at d(CA/TG) sequences? The value of $n_Z = 0.6$ for pCA/TG-36 most likely reflects not so much the number of spermines that bind to the plasmid insert but the negative cooperativity of ligand binding to this sequence. It is difficult to imagine less than 1 spermine bound per sequence.

The source of this negative cooperativity is likely associated with the B−Z transition in $d(CA/TG)_n$ sequences. The free energy required to propagate Z-DNA through d(CA/TG) dinucleotides has been determined to be 1.4 kcal mol^{-1} dn^{-1}. For a sequence of 18 d(CA/TG) dinucleotides, as in pCA/TG-36, the total $\Delta G_P°$ (25 kcal/mol) is greater than $\Delta G_N°$ (10 kcal/mol). Thus, the B−Z transition is not highly cooperative. Even at high negative superhelical densities ($\sigma \geq -0.05$), we would expect only a fraction of the dinucleotides in the insert to be in the Z form. This is consistent with results from chemical and nuclease probing studies showing that long d(CA/TG) sequences are not entirely Z-DNA in negatively supercoiled plasmids (Hayes & Dixon, 1985; Johnston *et al.*, 1988; Kladde *et al.*, 1994).

How does this affect spermine binding to Z-DNA? Consider a topoisomer with σ at the midpoint of the B−Z transition (where $\Delta G_{obs}°$ is defined). The $\langle \Delta Tw \rangle$ partitions through a d(CA/TG) insert according to the probability that each dinucleotide will form Z-DNA, but not uniformly (Figure 4A). The center of the sequence has a higher probability of forming Z-DNA than the ends. These central dinucleotides would therefore be the most probable sites for spermine binding. If spermine binds to ~10 bp of d(CA/TG), as with $d(CG)_n$ sequences, then the stabilization energy from ligation (approximately −11 kcal/mol) will primarily lower the propagation energy of the bound dinucleotides (by ~1.1 kcal mol^{-1} bp^{-1}). The probability that this region of the sequence will become Z-DNA is then significantly greater than that of the sequences that flank the binding site. Indeed, if the total amount of Z-DNA must remain constant (e.g., at 50% for the midpoint of the B−Z transition), the spermine bound to the central d(CA/TG) dinucleotides would necessarily result in a repartitioning of the conformation such that the dinucleotides flanking the binding site will become more B-like (Figure 4D).

We can simulate this effect by first defining a sequence of 18 dinucleotides of d(CA/TG) in which the $\Delta G_P°$ of the middle 10 base pairs has been reduced by 2.2 kcal mol^{-1} bp^{-1}. The statistical mechanics expressions (eqs 1 and 2) are then used to calculate the probability that any dinucleotide is in the Z form (P_{Z-DNA}) and the B form (P_{B-DNA}). This was calculated for σ at which 50% of the sequence is Z-DNA. The calculation was repeated for a standard set of d(CA/TG) dinucleotides for comparison. It is evident from this simulation that binding of a single spermine to the central 10 base pairs increases P_{B-DNA} of the dinucleotides that are outside of this binding site. In addition, the location of the B−Z junctions both with and without spermine are located 6 bp (three dinucleotides) from either end of the sequences. The B−Z junctions have been shown from nuclease digestion studies to be composed essentially of four unpaired nucleotides each (Sheardy *et al.*, 1994). Spermine has been shown to inhibit melting of the DNA duplex (Morgan *et al.*, 1986), suggesting that the B−Z junctions should have an even lower

affinity than even B-DNA for polyamines. Thus, binding the first spermine inhibits the binding of additional ligands because adjacent d(CA/TG) dinucleotides adopt conformations that have lower affinities for spermine. This does not mean that all other spermine molecules are excluded from binding to d(CA/TG) sequences, only that the affinities for subsequent binding steps are not as high as that for the first ligand.

This effect was not observed for pCG-24 because $\Delta G_P°$ for d(CG) dinucleotides (0.6 kcal mol^{-1} bp^{-1}) is very low compared to the nucleation term. The B−Z transition in this sequence is therefore highly cooperative, approaching the all-or-none limit. This means that, once there is sufficient energy from supercoiling to overcome the 10 kcal/mol for $\Delta G_N°$, the entire d(CG) insert uniformly converts to the Z form (Figure 4B,E). Spermine binding to a specific region of the insert does not dramatically affect the surrounding nucleotides because $\Delta G_P°$ for the bound and the unbound dinucleotides are both low and favor Z-DNA formation. To show that this effect is inherent to the dinucleotides and not the length of the sequence, this was repeated for 18 d(CG) dinucleotides. Again, Z-DNA is predicted to be uniformly distributed throughout the sequence with or without spermine (Figure 4C,F).

The expression derived for this study (eq 7) describes the effect of spermine binding to Z-DNA sequences in ccDNA. One can argue that the treatment of ligand binding to these inserts is too simplistic to describe the molecular details of the binding process. However, the greatest utility of the model is in its ability to accurately simulate and predict the ligand binding effects on the B−Z transition as it would likely be induced in the cell (*i.e.*, by negative supercoiling). The affinity constant and the number of spermines that bind to the mixed sequence pZ-40 can essentially be treated as a linear combination of the parameters for d(CG) and d(CA/TG) dinucleotides. Thus, the effect of spermine on the supercoil-induced B- to Z-DNA transition can be predicted for nearly any good Z-forming sequence. For example, we had previously located 329 potential Z-DNA-forming sequences from 98 genes (of 138 searched) in the human genome. The average sequence from this population can be defined as an 8-dinucleotide (16 bp) APP sequence composed of ∼6 d(CA/TG) and ∼2 d(CG) dinucleotides. Thus, the sequence d(TGTGTGTGCGCGTGTG) found in the promoter of the human cytokeratin 8 gene is representative of a typical Z-DNA-forming sequence in human genes. From our model, K_{ZP} for this average Z-DNA-forming sequence is predicted to be 5.4×10^7 M^{-1}, with $n_Z = 0.60$ spermine. Thus, $\Delta G_{B-Z}° = 19.2$ kcal/mol in the absence of ligand and is predicted to be reduced by 1.8 kcal/mol to 17.4 kcal/mol at 5 μM spermine but will return to near its starting value of ∼19 kcal/mol at 30 μM spermine (Figure 5A). In terms of superhelical density, the midpoint of the B−Z transition occurs at $\sigma = -0.07$ in the absence of spermine, will be reduced to −0.063 at 5 μM spermine, and will return to −0.07 at 30 μM spermine (Figure 5B,C).

How does this relate to conditions typically found in eukaryotic cells? The best estimate for the superhelical density in the cell is $\sigma \cong -0.06$ (Bauer, 1978). Thus, the average Z-forming sequence such as the one found in the cytokeratin 8 gene is in the B form at this superhelical density but can be induced to Z-DNA by a small amount of free spermine (∼5 μM). How much spermine is in the cell's nucleus? One estimate is that there is 1.4 nmol of spermine in the nuclei of 10^6 BSC-1 cells during exponential growth (Mach *et al.*, 1982). This translates to ∼300 μM in the nucleus (assuming a 10 μm nucleus). This likely overestimates the free polyamine concentration since much of this will be bound to the nucleic acids of the nuclei. The concentration of DNA binding sites in a cell is ∼200 μM. The presence of RNA would dramatically increase the number of potential binding sites. Bayers *et al.* (1992) had shown that ∼2 μM spermine in the growth media can rescue murine leukemia L1210 cells whose growth had been arrested by exposure to 5′-[[[(*Z*)-4-amino-2-butenyl]methyl]amino]-5′-deoxyadeosine (AbeAdo). AbeAdo inhibits *S*-adenosyl-l-methionine decarboxylase which catalyzes the rate-limiting step in spermine and spermidine biosynthesis, thereby reducing their levels in the exposed cells to below detectable limits. This suggests that the level of free spermine required to sustain normal growth in a eukaryotic cell is on the order of ∼2 μM.

The average Z-DNA-forming sequence in the human genome is thus highly sensitive to the level of supercoiling and spermine in the cell's nucleus, and these levels appear to be in the range where the B−Z transition is most sensitive to both factors. Any small change in either will significantly influence the conformation of these sequences. For example, the increase in negative superhelicity generated in the wake of a transcribing RNA polymerase (Liu & Wang, 1987) would favor Z-DNA (Rahmouni & Wells, 1989) in a

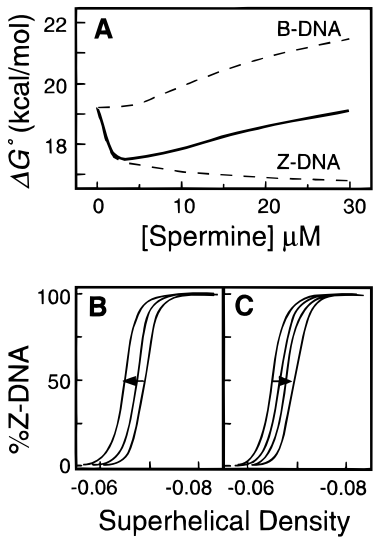

FIGURE 5: Effect of spermine on the B−Z transition as predicted for d(TGTGTGTGCGCGTGTG). (A) The effect of increasing spermine concentratrions on the transition free energy ($\Delta G°$) was calculated using eq 7 in the text. $K_{ZP} = 5.5$ M^{-1}, $n_Z = 0.54$ spermine, $K_{BP} = 1.4 \times 105$ M^{-1}. $n_B = 2.6$ spermines. The solid line represents the overall change in $\Delta G°$ predicted, while the dashed lines represent the effect of spermine on $\Delta G°$ from binding to only the Z form or only the B form of the sequence, as labeled. (B) The effect of low spermine concentrations (0, 1, and 5 μM) on the B−Z transition as simulated by the statistical mechanics treatment of the zipper model (eqs 1 and 2 in the text). Curves are the percent Z-DNA as defined from the $\langle \Delta Tw \rangle$ calculated from eq 2 versus superhelical density (calculated as the number of supercoils per turn of B-DNA). The arrow indicates the direction in which spermine shifts the midpoint of the transition (to lower superhelical densities). (C) The effect of high spermine concentrations (5, 10, 20, and 30 μM) on the B−Z transition as simulated by the statistical mechanics treatment of the zipper model. The arrow indicates the direction in which spermine shifts the midpoint of the transition (to higher superhelical densities).

sequence such as that found in the promoter of the cytokeratin 8 gene. Spermine levels are also known to be highly dependent on the cell cycle [reviewed in Tabor and Tabor (1984)], which may induce Z-DNA formation at low levels or reverse its formation at higher levels as may be required. As both these factors become better defined, the model presented here and the parameters determined from this model will be useful for predicting the DNA conformation during various functional periods of the cell cycle.

ACKNOWLEDGMENT

We thank Dr. Peter Yau for providing topoisomerase, the Protein and Nucleic Acids Service Core (under the direction of Prof. M. Schimerlik) of the Environmental Health Sciences Center at Oregon State University for help in data analysis, Blaine Mooers for his assistance, and the entire Ho lab for many helpful discussions.

REFERENCES

Bancroft, D., Williams, L. D., Rich, A., & Egli, M. (1994) *Biochemistry 33*, 1073−1086.
Basu, H. S., & Marton, L. J. (1987) *Biochem. J. 244*, 243−246.
Basu, H. S., Feuerstein, B. G., Zarling, D. A., Shafer, R. H., & Marton, L. J. (1988) *J. Biomol. Struct. Dyn. 6*, 299−309.
Bauer, W. R. (1978) *Annu. Rev. Biophys. Bioeng. 7*, 287−313.
Behe, M., & Felsenfeld, G. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 1919−1923.
Braunlin, W. H., Strick, T. J., & Record, M. T., Jr. (1982) *Biopolymers 21*, 1301−1314.
Byers, T. L., Ganem, B., & Pegg, A. E. (1992) *Biochem. J. 287*, 717−724.
Depew, R. E., & Wang, J. C., (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 4275−4279.
Droge, P. (1994) *BioEssays 16*, 91−99.
Ellison, M. J., Kelleher, R. J., III, Wang, A. J., Habener, J. F., & Rich, A. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 8320−8324.
Feuerstein, B. G., Williams, L. D., Basu, H. S., & Marton, L. J. (1991) *J. Cell. Biochem. 46*, 37−47.
Freeman, L. A, & Garrard, W. T. (1992) *Crit. Rev. Eukaryotic Gene Expression 2*, 165−209.
Fuller, F. B. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 815−819.
Geierstanger, B., Kagawa, T. F., Quigley, G. J., & Ho, P. S. (1991) *J. Biol. Chem. 266*, 20184−20191.
Gross, D. S., & Garrard, W. T. (1986) *Mol. Cell. Biol. 6*, 3010−3013.
Hamada, H., & Kakunaga, T. (1982a) *Nature 302*, 632−635.
Hamada, H., Petrino, M. G., & Kakunaga, T. (1982b) *Proc. Natl. Acad. Sci. U.S.A. 79*, 6465−6469.
Hayes, T. E., & Dixon, J. E. (1985) *J. Biol. Chem. 260*, 8145−8156.
Hirschmann, S. Z., Leng, M., & Felsenfeld, G. (1967) *Biopolymers 5*, 227−233.
Ho, P. S. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 9549−9553.
Johnston, B. H., Ohara, W., & Rich, A. (1988) *J. Biol. Chem. 263*, 4512−4515.
Jovin, T. M., Soumpasis, D. M., & McIntosh, L. P. (1987) *Annu. Rev. Phys. Chem. 38*, 521−560.
Kladde, M. P., Kohwi, Y., Kohwi-Shigematsu, T., & Gorski, J. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 1898−1902.
Liu, L. F., & Wang, J. C. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 7024−7027.
Mach, M., Ebert, P., Popp, R., & Ogilvie, A. (1982) *Biochem. Biophys. Res. Commun. 104*, 1327−1334.
McGhee, J. D., & von Hippel, P. H. (1974) *J. Mol. Biol. 86*, 469−489.
Mclean, M. J., Blaho, J. A., Kilpatrick, M. W., & Wells, R. D. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 5884−5888.
Mirkin, S. M., Lyamichev, V. I., Kumarev, V. P., Kobzev, V. F., Nosikov, V. V., & Vologodskii, A. V. (1987) *J. Biomol. Struct. Dyn. 5*, 79−88.
Morgan, J. E., Blankenship, J. W., & Matthews, H. R. (1986) *Arch. Biochem. Biophys. 246*, 225−232.
Peck, L. J., & Wang J. C. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 6206−6210.
Peck, L. J., Nordheim, A., Rich, A., & Wang, J. C. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 4560−4564.
Rahmouni, A. R., & Wells, R. D. (1989) *Science 246*, 358−363.
Rich, A., Nordheim, A., & Wang, A. H.-J. (1984) *Annu. Rev. Biochem. 53*, 791−846.
Schroth, G. P., Chou, P.-J., & Ho, P. S. (1992) *J. Biol. Chem. 267*, 11846−11855.
Sheardy, R. D., Levine, N., Marotta, S., Suh, D., & Chaires, J. B. (1994) *Biochemistry 33*, 1385−1391.
Tabor, C. W., & Tabor, H. (1984) *Annu. Rev. Biochem. 53*, 749−794.
Thomas, T. J., & Thomas, T. (1994) *Biochem. J. 298*, 485−491.
Thomas, T. J., Gunnia, U. B., & Thomas, T. (1991) *J. Biol. Chem. 266*, 6137−6141.
Vologodskii, A. V., & Frank-Kamenetskii, M. D. (1984) *J. Biomol. Struct. Dyn. 1*, 1325−1333.
Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G., & Rich, A. (1979) *Nature 282*, 680−686.
Wittig, B., Dorbic, T., & Rich, A. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 2259−2263.

BI961881I