Effects of A:T base pairs on the B-Z conformational transitions of DNA

Fu-Ming Chen

Department of Chemistry, Tennessee State University, Nashville, TN 37209-1561, USA

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ABSTRACT

Effects of A:T base pairs on the propensity of B to Z conformational transitions have been investigated by the CD salt titrations on d(CG)₅, d(GC)₅, terminal or central A:T replaced decamers, and terminal A:T appended dodecamers. The presence of A:T at the center greatly inhibits the B to Z transition of both G:C decamers. Moderate Z inhibitions are shown by terminal A:T replacements and additions to d(CG)₅, with the former exhibiting a stronger effect. In contrast, the addition and replacement with A:T at the terminals of d(GC)₅ facilitate the B to Z conversion, with the replacement exhibiting a somewhat more pronounced effect. These results may be rationalized in terms of the number of contiguous CG sequences present in an oligomer and the relative inhibitory effects of other dinucleotide sequences. Our results also suggest that some short oligomers with purine at the 5'-end, such as d[A(CG)_nT] with $n \geq 2$, may likely crystallize as Z conformations.

INTRODUCTION

DNA conformational transitions are of interest as they may be related to the gene regulatory processes. Such interests have further been heightened by the discovery in 1979 of left-handed Z DNA (1). Factors affecting the B-Z interconversions have since been extensively investigated (2). Most of these studies, however, have been carried out with poly(dG-dC):poly(dG-dC), poly(dG-m⁵dC):poly(dG-m⁵dC) and their fragments. The occurrence of a long alternating dC-dG stretch is known to be relatively rare in native DNA and thus studies on the effect of A:T base pairs on the B-Z transitions will be of value.

Although there have been some studies made on the effect of A:T base pairs on the B to Z conformational transitions, few systematic investigations were made until very recently. Quadrifoglio et al. (3) noted that when (dC-dG)₃ is flanked by three alternating AT sequences the left-handed helix is unstable and a B-type

conformation is obtained even at high salt conditions. Pohl (4) mentioned that solution studies with d(CGCGATGCG) indicate that the introduction of alternating A-T base pairs leads to an unfavorable free-energy change of at least 5 kJ/mole base pair. Although some short $(dC-dG)_n$ and $(m^5dC-dG)_n$ with A:T base-pair inserts have been found to be capable of existing in Z conformations in crystals (5,6,7,8), it was noted that AT base pairs are less stable than GC base pairs in Z-DNA (6). Indeed, most of these oligomers cannot be completely converted to the Z-form in aqueous solutions even at very high salt conditions. Cavailles et al. (9) have noted that the B-Z transitions of oligonucleotides are highly base sequence dependent and the replacement of only two residues by dT and dA in a relatively long sequence is enough to strongly destabilize the Z form. Schorschinsky & Behe (10) have synthesized poly[d(CGCGCGTGCA)], an alternating purine-pyrimidine polymer with a 10-base pair repeating unit. It was shown that this polymer can undergo a right-left-hand helical transition, but the conformational transition requires a much higher salt concentration than with poly(dG-dC):poly(dG-dC). Most recently, Wang et al. (11) have carried out a laser Raman study on a series of self-complementary octamers containing all four deoxynucleotide bases in order to determine the sequence dependence of the B to Z transition in crystals and in aqueous NaCl solutions. Their results will be discussed in more detail in the "Discussion" section.

The purpose of this paper is to investigate the effect of A:T base pairs on the B-Z conformational transitions in a more systematic fashion. Salt titrations were carried out with d(CGCGCGCGCG) and d(GCGCGCGCGC) as well as decamers obtained by replacing the terminal or central base pairs with A:T base pairs. Comparisons with dodecamers obtained by adding A:T base pairs at both ends while maintaining the purine-pyrimidine alternating feature have also been made. Effects of temperature and methanol on the Z forming propensity of these oligomers at high salt conditions will also be investigated.

MATERIALS AND METHODS

Deoxyoligonucleotides were synthesized with a Biosearch 8600 DNA Synthesizer using the beta-cyanoethyl phosphoramite chemistry

(12). The detritylated products were subsequently purified by anion exchange and C-18 reverse phase HPLC. The lyophilized oligomers were then dissolved in 10 mM tris-HCl buffer of pH 8 containing 0.1 M NaCl. Concentrations of these oligonucleotides (per nucleotide) were determined with absorbances at 260 nm after melting, using extinction coefficients listed below.

Oligonucleotide	Extinction coefficient (per base)
d(CGCGCGCGCG)	8,480 M ⁻¹ cm ⁻¹
d(TGCGCGCGCA)	8,900
d(CGCGTACGCG)	9,100
d(ACGCGCGCGCGT)	8,930
d(GCGCGCGCGC)	8,440
d(ACGCGCGCGT)	9,040
d(GCGCATGCGC)	8,860
d(TGCGCGCGCGCA)	8,810

These extinction coefficients were obtained through nearest neighbor approximation using mono- and di-nucleotide values tabulated in the Handbook of Biochemistry and Molecular Biology.

Absorption spectra were measured with a Cary 210 Spectrophotometric System. CD spectra were measured by a JASCO J-500A Recording Spectropolarimeter at appropriate temperatures using a water-jacketed cell of 2-cm pathlength. Salt titrations were carried out with oligomer concentrations of 40 μ M (per nucleotide). Temperatures were maintained by a Neslab RTE-8 refrigerated circulating bath. Desired NaCl concentrations were obtained by adding appropriate amounts of solid NaCl into the solution. A 2% volume increase per molar NaCl added was used in the concentration correction. This value had been determined from a separate experiment.

RESULTS

Salt Titrations

Comparison Between d(CGCGCGCGCG) and d(GCGCGCGCGC). Representative CD spectra of d(CG)₅ and d(GC)₅ at several NaCl concentrations are shown in Figures 1A and 1B. As expected, the decamer starting with cytosine at the 5' end is more easily converted to the Z conformation. For d(CG)₅, the B to Z transition midpoint is roughly 2.2 M NaCl (since most of the oligomers studied do not achieve complete Z conformation even at 4.6 M NaCl, all transition mid-

Figure 1. Comparison of representative CD spectra during salt titrations for d(CGCGCGCGCG) (A), d(GCGCGCGCGC) (B). Measurements were made at room temperature. Appropriate amounts of NaCl were directly added to the solution contained in a 2-cm pathlength cylindrical cell and dissolved by rigorous shaking. Ellipticity at 293 nm was monitored after each NaCl addition and the spectrum was then measured after the equilibrium had been reached.

points have been estimated with the salt concentrations at which the 293 nm ellipticities change from + to -) and the conversion to the Z form is nearly complete at 4.6 M, as judged by the 293 and 253 nm ellipticities (Figure 1A). In contrast, the characteristic negative 293 nm CD band of Z-DNA is not evident for d(GC)₅ even at 4.6 M NaCl (Figure 1B). Some conformational transitions, how-

ever, are indicated by significant spectral alterations in this region for $d(GC)_5$.

It is interesting to note that spectral alterations resulting from salt titrations below 2.4 M NaCl consist mainly of intensity reductions at the 280 nm region without the corresponding decrease at the 253 nm band and, consequently, no isoelliptic point is present. For titrations at higher salt concentrations, however, the isoelliptic points are readily apparent. Additional conformational transitions prior to the onset of the B-Z interconversions at high salt are, thus, suggested.

Effects of Central and Terminal Base Pair Replacements. Replacement with the A:T base pairs at the center greatly inhibits the Z formation for both oligomers, as is indicated by the absence of 293 nm negative ellipticities even at 4.6 M NaCl. In fact, the spectra at this high salt concentration are characteristic of B form, which are not very different from the corresponding 0.1 M NaCl solutions (results not shown).

Effects on the terminal base replacement are shown in Figures 2A and 2B. Although the terminal replacement with A:T base pairs in $d(CG)_5$ appears to also inhibit the Z formation (compare Figures 1A and 2A), its effect is much less than that of central replacement. In contrast to the central A:T base-pair replacement, however, significant CD spectral alterations are evident upon salt titrations, including a weak negative 293 nm CD band in the 4.6 M NaCl solution (Figure 2A). A B-Z transitional midpoint of 4.4 M NaCl has been estimated. In contrast, terminal replacements with A:T base pairs in $d(GC)_5$ greatly facilitate the B to Z transformation (compare Figures 1B and 2B). As can be seen in Figure 2B, more than 70% conversion to the Z form has occurred at 4.6 M NaCl, judging from the 253 nm CD intensities, and a transition midpoint of 3.3 M NaCl (as compared to > 4.6 M for the parent decamer) can be estimated. It is also noteworthy that this transition midpoint salt concentration is lower than that observed with terminal replacements of $d(CG)_5$ (compare Figures 2A and 2B).

The spectral alterations for these A:T containing decamers, including the absence of an isoelliptic point during lower salt titrations and the appearance of an isoelliptic point at higher

Figure 2. Representative CD spectra during NaCl titrations for d(TGCGCGCGCA) (A) and d(ACGCGCGCGT) (B), obtained from terminal A:T replacements of d(CG)₅ and d(GC)₅, respectively.

salt titrations, are similar to those noted earlier for the parent decamers.

Effect of A:T Base-pair Addition at the Terminals. The addition of A:T base pairs at both ends of d(CG)₅ results in only a slight inhibition of B to Z transition as suggested by the increase of the transition midpoint from 2.2 to 2.7 M NaCl. Such inhibition is much less pronounced than that due to terminal replacements. Interestingly, the terminal addition to d(GC)₅, on the other hand, moderately facilitates the Z formation (transition midpoint

Table I.
Comparison of Approximate B-Z Transition Midpoints.

Oligomer	B-Z Midpoint ^a	Oligomer	B-Z Midpoint ^a
d(CGCGCGCGCG)	2.2 M NaCl	d(GCGCGCGCGC)	> 4.6 M NaCl
d(CGCGTACGCG)	>> 4.6	d(GCGCATGCGC)	>> 4.6
d(TGCGCGCGCA)	4.4	d(ACGCGCGCGT)	3.3
d(ACGCGCGCGCGT)	2.7	d(TGCGCGCGCGCA)	4.0

^aApproximated with the NaCl concentration at which the ellipticity at 293 nm changes from positive to negative.

of 4.0 M NaCl as compared to > 4.6 M), somewhat less effectively than the terminal replacement which exhibits a transition midpoint of 3.3 M NaCl.

For comparison, the B-Z transition midpoints for the aforementioned decamers and dodecamers are summarized in Table I.

Thermal Effect

In all of the oligonucleotides studied, the Z form appears to be favored at low temperatures under high NaCl concentrations. A similar temperature effect has previously been observed (3). Such thermal effects are illustrated in Figures 3A and 3B for the decamer d(TGCGCGCGCA) and the dodecamer d(TGCGCGCGCGCA), respectively. Judging from the progressive enhancement of the 293 nm negative ellipticity, a significant increase in the Z form is seen for both oligomers in 4.6 M NaCl when the temperature is lowered from 30 to 0 °C. A sizable negative 293 nm intensity becomes apparent for d(TGCGCGCGCA) at 0°C even though it is barely discernable at 30 °C (Figure 3A). Comparison of these two figures also serves to illustrate the fact that T(GC)₅A is more easily converted to the Z form than T(GC)₄A.

Effect Due to Methanol

Alcohol has been known to stabilize the Z conformation (13). The addition of methanol to the oligomer solutions containing high salt can, thus, further facilitate conformational transitions. This is illustrated with d(GC)₅ as shown in Figure 4A. In the absence of MeOH, lowering the temperature has only negligible effect on the conformation of d(GC)₅ in a 4.6 M NaCl solution (not shown). Addition of methanol, however, significantly alters the CD spectra and results in a progressive enhancement of the 293 nm negative

Figure 3. Effect of temperature on the CD spectra of decamer d(TGCGCGCGCA) (A) and dodecamer d(TGCGCGCGCGCA) (B) in 4.6 M NaCl solutions.

ellipticity and a concomitant intensity reduction at the 253 nm band, yielding an isoelliptic point at around 280 nm.

The combined effect of temperature and methanol is illustrated with the decamer d(CGCGTACGCG) as shown in Figure 4B. As indicated by the absence of the 293 nm negative band, this polymer exhibits predominantly a B conformation in 4.6 M NaCl solution. Although decreasing the temperature from 40 to 2 °C significantly reduces the positive intensity around the 280 nm region, no 293 nm negative ellipticity is evident and a negligible effect on the 253 nm band is seen. Lowering the temperature in the presence of high

Figure 4. (A): Representative CD spectra of d(GCGCGCGCGC) in solutions containing various amounts of MeOH and nearly saturating amounts of NaCl. (A): Effect of temperature on the CD spectra of d(CGCGTACGCG) in the presence and in the absence of MeOH. All solutions contain nearly saturating amounts of NaCl.

salt and methanol, however, results in a significant Z contribution as evidenced by the appearance of a moderately strong negative 293 nm CD band. No negative 293 nm CD band is apparent for the decamer d(GCGCATGCGC) even under these extreme conditions.

DISCUSSION

Most recently, Wang et al. (11) carried out a Raman study on the propensity of Z formation in a series of self-complementary

pyrimidine-purine alternating octameric deoxynucleotides containing one A and one T. They rationalized their results in terms of the relative stability of pyrimidine-purine dinucleotide units in the Z form with the order $(CG) > (TG) = (CA) > (TA)$ and the added stability of sequential (CG) units. Our results appear to be in general agreement with such an analysis and add further insights into the inhibitory effect of purine-pyrimidine dinucleotide sequences.

Our titration results indicate a much easier B to Z transition for $d(CG)_5$ than for $d(GC)_5$. This is consistent with earlier observations (3,14) suggesting that a GC alternating oligomer is more difficult to enter into the Z conformation if it begins with guanine at the 5' end. Since there are 5 consecutive CG sequences in $d(CGCGCGCGCG)$ while only 4 are present in $d(GCGCGCGCGC)$ with one additional GC sequence, our results, thus, appear to agree with the interpretation that the longer the consecutive dC-dG stretch, the easier and more cooperative the B to Z transition. However, the fact that an octamer $d(CGCGCGCG)$ can be completely transformed into a Z conformation with saturating amounts of NaCl (11), whereas the decamer $d(GCGCGCGCGC)$ cannot, also suggests that the terminal GC dinucleotide sequences strongly inhibit Z formation.

The fact that the replacement of central or terminal base pairs with the A:T base pairs in $d(CG)_5$ results in an inhibition of the B to Z transition, with the central replacement being much more effective, can also be rationalized in terms of the length of contiguous CG stretch. The reduction from 5 consecutive CG sequences in $d(CGCGCGCGCG)$ to 3 in $d(TGCGCGCGCA)$ and 2 in $d(CGCGTACGCG)$ appears to account for their progressive inhibition on the Z formation. Since no reduction in the consecutive CG sequences occurs upon adding A:T base pairs at the terminals of $d(CG)_5$, the slight B to Z transitional inhibition in $d(ACGCGCGCGCGT)$ may be attributed to the slight inhibitory action of the terminal dinucleotide AC (GT) sequences. The greater inhibitory action of terminal replacement compared to terminal addition on $d(CG)_5$ is also accounted for by the reduction of two CG sequences upon terminal replacement with A:T base pairs.

Although the central A:T base-pair replacement of $d(GC)_5$ also greatly inhibits the Z formation, the terminal base-pair replace-

ment results in a significant facilitation of the B to Z transition. The transition midpoint for d(ACGCGCGCGT) is now decreased to around 3.3 M NaCl from more than 4.6 M. Interestingly, such B to Z facilitation is also observed with the addition of A:T base pairs at the terminals, albeit slightly less effective. The transition midpoint for the dodecamer d(TGCGCGCGCGCA) is around 4.0 as compared to > 4.6 M NaCl for d(GC)₅. The B to Z transitional inhibition upon central base-pair replacement can be understood in terms of the reduction of consecutive CG sequences from 4 to 1 in d(GCGCATGCGC). Since the number of consecutive CG sequences remains the same, the facilitation of B to Z transition upon terminal replacement to form d(ACGCGCGCGT) may be the consequence of the fact that inhibition of Z formation due to the terminal AC (CT) sequence is much less effective than the terminal GC sequence. The moderate B to Z transitional facilitation upon terminal base-pair addition may be the consequence of a slight Z favoring of the TG (CA) sequence or a slight increase in transitional cooperativity due to chain length increase.

The Z inhibitory effect of the terminal GC sequence appears to be also supported by our observation that decamer d(GCGCATGCGC) cannot be converted to the Z form at 0 °C by adding MeOH to the solution containing saturating amounts of NaCl whereas it was reported that octamer d(CGCATGCG) can be converted to Z form under roughly similar conditions (11). The stronger inhibitory effect of the G-C dinucleotide sequence as compared to A-C (G-T) may be the result of end effects, such as easier fraying for A:T, since it has been found that while d(CGCATGCG), which has two internal G-C sequences, can be converted to the Z form under extreme conditions, while d(CGTGCACG), with only one G-C, cannot (11).

In summary, our results revealed that although the propensity for the B to Z conformational transition is dominated by the length of a (CG)_n stretch in DNA, it is also significantly modulated by the nucleotide base flanking this stretch on the 5'-end, with guanine exhibiting greater inhibitory effect than adenine. The ability for A(CG)₅T and A(CG)₄T to achieve Z conformation at high salt as shown by our studies is particularly noteworthy, since all the Z crystal structures solved thus far have been with oligomers beginning with pyrimidine. The fact that few attempts have been

made to crystallize short alternating oligomers with purine at the 5'-end may be due to the popular belief that only short oligomers beginning with pyrimidine can be converted to the Z form. Since some oligomers which cannot be converted into Z form in solution have been found to crystallize in Z conformation, it is reasonable to expect that these two oligomers, and maybe even d(ACGCGCGT), with purine at the 5'-end will also exhibit Z conformation in crystal.

Finally, it should be pointed out that our studies of short oligomers entail the end effects. Thus, the relevance of these results to the B-Z transitions of similar sequences embedded in genomic DNA may be somewhat clouded and further clarification with longer oligomers will be required. It should also be noted that Wang et al. (15) had just published Raman results on d(ACGCGCGT) indicating a Z conformation in crystalline state as well as in a solution of saturated NaCl/20% EtOH. Their observations are consistent with and further strengthen our conclusions. The same authors (15) had also examined the decamer d(CGCGTACGCG) in solution and reported that at saturated NaCl and 2 °C a 67% conversion to the Z form was observed. No concentration dependence on the degree of conversion was observed in a limited concentration range studied (0.5 to 2.0 OD/ μ L). The degree of Z conversion for this oligomer reported in our studies, however, is minimal under comparable conditions. This apparent discrepancy may be accounted for by the possible hairpin formation of this oligomer. Indeed, hairpin formation with a loop containing only two bases had earlier been reported (16). Our melting experiments with absorbance and CD at different oligomer concentrations have confirmed the existence of hairpin formation for this oligomer (results not shown). At the low oligomer concentration (40 μ M in nucleotide or 4 μ M in strand) used in our experiments, the hairpin conformations are predominant and do not favor Z formation, as only two consecutive CG sequences are present in such a hairpin. At the high oligomer concentrations (about 1000-fold more concentrated than ours) used by Wang et al. (15), the duplex forms are favored and consequently easier Z formation at high salt conditions, as two CGCG stretches are present in such a duplex. It is reasonable to suspect that

hairpin formation may also play a role in the B-Z transitions of the sequence isomer d(GCGCATGCGC).

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