Conformation and dynamics of Z-DNA oligomer duplex of d[(CG)₃TATA(CG)₃] in solution

Satoshi Ikuta* and Yu-Sen Wang

Department of Chemistry, Illinois Institute of Technology, Chicago, IL 60616, USA

ABSTRACT
The conformation and dynamics of a DNA oligomer, d[(CG)₃TATA(CG)₃], in 4M NaClO₄ (Z-
TATA 16 mer) have been studied by ¹H NMR. The principal results of our investigation are: (i)
at low temperature d[(CG)₃TATA(CG)₃] exists as duplexes in both low (0.1M NaCl) and high (4M
NaClO₄) ionic strength solutions; (ii) CGCGCG segments undergo the B-to-Z transition in 4M
NaClO₄; (iii) even in 4M NaClO₄ the TATA box exhibits non-Z-structures and possesses multiple
conformations which are slowly exchanging on the NMR chemical shift difference time scale; and,
(iv) the Z-type structure of the CGCGCG segments induced in 4M NaClO₄ is more conformationally
mobile than its B-type counterpart in 0.1M NaCl on the nanosecond time scale.

INTRODUCTION
The discovery of the left-handed Z-DNA (1-3) suggests that this structure plays an
important role in vivo. For example, Z-DNAs are often found in supercoiled plasmids
under physiological conditions (4,5), and Z-DNA binding proteins are also found in a diverse
group of cells (6,7). Through many independent studies, it has become clear that the
conformation and dynamics of DNA molecules are determined by the nature of the bases,
their sequences, and environments. In vivo, Z-type conformations are formed in various
alternating purine-pyrimidine sequences including dA·dT base pairs (8-10). We have
chosen to apply spectroscopic techniques to a study of the physical properties of short
DNA oligomers in vitro as a model for in vivo systems.

Insertion of dA·dT base pairs into dG·dC stretches in DNA oligomers often prevents
the oligomers from exhibiting Z-type structures. For example, the structure and dynamics
of a self-complementary d[(CG)₃TATA(CG)₃] duplex were studied by ¹H and ³¹P NMR to
monitor the potential for Z-DNA formation (11). In that study there was, however,
no evidence of the B-to-Z transition for the 16 mer, even in saturated NaCl solution, except
for a minor peak in the ³¹P NMR spectrum corresponding to about 11% of the molecules
in the Z-form. The conclusion was drawn that the incorporation of a central TATA segment
in the alternating CG 16 mer inhibits formation of the Z-helix (11).

In contrast, we have found that 4M NaClO₄ converts the right-handed helix (B-TATA
16 mer) into the left-handed helix (ZTATA 16 mer). The structure and dynamics of B-
and Z-TATA 16 mer were investigated by one- and two-dimensional NMR techniques.
The dynamics of B- and Z-TATA 16 mer are compared with B- and Zd(CG)₃.
Nucleic Acids Research

Figure 1. Sequence of d[(CG)3TATA(CG)3] is shown with the numbering system used.

**EXPERIMENTAL**

**Materials**

DNA oligomers, d[(CG)3TATA(CG)3] and d(CG)3, were synthesized by the phosphotriester method (12). After the synthesis, the desired DNA oligomers were purified by the procedure described previously (13). TATA 16 mer (73 OD units at 260 nm) was lyophilized twice in D2O and dissolved in 0.3 ml of 99.995% D2O (Stohler Isotopes), containing 10 mM phosphate buffer and 0.1M NaCl at pH=6.6 (B-TATA 16 mer). For the preparation of a high ionic strength sample (Z-TATA 16 mer) dried NaClO4 was directly added to a low ionic strength sample (B-TATA 16 mer). Similarly, B-type d(CG)3 [B-d(CG)3] and Z-type d(CG)3 [Z-d(CG)3] were prepared using 73 OD units of hexamer.

**NMR Measurements**

NMR spectra and pre-steady state NOE were obtained on a Nicolet NT-300 instrument. One-dimensional (1-D) Nuclear Overhauser Effects were recorded by difference spectroscopy with presaturation pulses of defined time duration noted in the figure caption. The cross-relaxation rates were obtained from the initial build-up profiles of NOEs.

Pure absorption phase NOESY spectra were obtained with 2048 points in t2 and 256 points in t1 (14). 64 scans were accumulated with 3 s relaxation delays between acquisitions. COSY spectra were recorded with 1024 points in t2 and 512 points in t1. Two-dimensional (2-D) data were processed on the VAX-780 computer by using the HARE program (from Dr. D. Hare). Chemical shifts (ppm) were determined relative to the chemical shifts of the residual HOD peak in 0.1M NaCl and 4M NaClO4 which were calibrated by the signal of DSS (2,2-dimethyl-2-silapentane-5-sulfonate).

**Circular Dichroism Measurements**

Circular dichroism spectra were obtained at 5°C on a custom built vacuum uv spectropolarimeter. CD spectra were recorded from 230 nm to 310 nm.

**UV Melting**

UV melting was measured using the methods and apparatus described elsewhere (15).

**RESULTS**

The sequence of d[(CG)3TATA(CG)3] along with the numbering system used are shown in Figure 1. The most significant findings are: (i) at low temperature, TATA 16 mer exists as a duplex in both low (0.1M NaCl) and high (4M NaClO4) ionic strength solutions; (ii) CGCGCG segments of TATA 16 mer undergo the B-to-Z transition in 4M NaClO4; (iii) even in 4M NaClO4 the TATA box exhibits non-Z- structures and possesses multiple conformations which are slowly exchanging on the NMR chemical shift difference time scale; and (iv) The Z-type structure of the CGCGCG segments induced in 4M NaClO4 is more conformationally mobile than B-type counterpart in 0.1M NaCl on the nanosecond time scale.

**Biphasic Transition of B- and Z-TATA 16 mer**

Figure 2 shows the differential melting curves obtained from absorbance measured at 268 nm as a function of temperature for Band Z-TATA 16 mer at an oligomer concentration.
Figure 2. Differential melting curves of B- TATA 16 mer in 0.1M NaCl and Z-TATA 16 mer in 4M NaClO₄ solutions.

of 5 μM. The biphasic transitions are the most important feature of the melting curves for B- and Z-TATA 16 mer. This behavior is in contrast to the monophasic melting transition of B- and Z-d(CG)₃ (16), but is in good agreement with the biphasic transitions observed for d[(CG)₃T5(CG)₃] (17) and d[(CG)₃TA(CG)₃] (18).

B-TATA 16 mer melts with transition temperatures (Tms') of 43 and 84°C while Tms' of Z-TATA 16 mer are 28 and 68°C. The Tms' of Z-TATA 16 mer are ~15°C lower than the corresponding Tms' of B-TATA 16 mer which indicates that high ionic strength (4M NaClO₄) destabilizes both duplexes and hairpins in B- and Zforms to a similar extent. Values of Tms for B- and Z-TATA 16 mer and B- and Z-d(CG)₃ are summarized in Table I. The biphasic transitions observed are consistent with the early NMR observation of loop formation in d[CG]₂TATA(CG)₂ (TATA 12 mer) duplex on decreased oligomer and counterion concentrations (11,19). These results indicate that (i) below the first Tm, Band Z-TATA 16 mer predominantly exist as a duplex; (ii) as the temperature is raised, a transition (the first Tm) from the duplex to a unimolecular hairpin occurs; and (iii) further increase in temperature results in a second transition (the second Tm) from a hairpin to a coiled single strand.

Circular Dichroism (CD) Spectra

The CD spectra of B- and Z-TATA 16 mer in 0.1M NaCl and 4M NaClO₄ are shown in Figure 3. In 0.1M NaCl, the CD spectra appear to be typical for B-like structures, with a negative peak at 255 nm, a positive peak at 280 nm, and a zero point at 266 nm. In
**Table I.** Transition Midpoints (Tm, °C) obtained by the UV melting experiments at 268 nm for TATA 16 mer and d(CG)$_3$ in 0.1 M NaCl and 4.0 M NaClO$_4$ solutions.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>1st Tm</th>
<th>2nd Tm</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-TATA 16 mer</td>
<td>0.1 M NaCl</td>
<td>43</td>
</tr>
<tr>
<td>Z-TATA 16 mer</td>
<td>4 M NaClO$_4$</td>
<td>28</td>
</tr>
<tr>
<td>B-d(CG)$_3$</td>
<td>0.1 M NaCl</td>
<td>43</td>
</tr>
<tr>
<td>Z-d(CG)$_3$</td>
<td>4 M NaClO$_4$</td>
<td>24</td>
</tr>
</tbody>
</table>

4 M NaClO$_4$, an inverted, red shifted CD spectrum is observed with a positive maximum at 275 nm, a zero point at 289 nm and a negative peak at 296 nm. The inversion of the CD spectra in 4 M NaClO$_4$ is consistent with our NMR observation that the guanine residue adopts the syn conformation around the β-glycoside bond, and, therefore, exists predominantly in the Z-form (*vide infra*).

**Spectra of B- and Z-TATA 16 mer**

NMR spectra of B- and Z-TATA 16 mer at 5°C are presented in Figures 4A and 4B, respectively, along with proton assignments. Both B- and Z-TATA 16 mer exhibit similar spectra. The principal differences are: (i) two signals corresponding to the two AH8 are observed in B-TATA 16 mer but at least six signals are seen in Z-TATA 16 mer (marked by *1); (ii) the sharp GH1' signal characteristic of Z-DNA (20) is seen at 6.19 ppm (marked by *2); (iii) two signals corresponding to the two T-CH$_3$ are observed in B-TATA 16 mer while multiple signals (see below) are observed in Z-TATA 16 mer (marked by

---

Figure 3. CD spectra of (A) B-TATA 16 mer in 0.1 M NaCl and (B) Z-TATA 16 mer in 4 M NaClO$_4$ solutions.
*3); and (iv) the H2' or 2" for the Z-TATA 16 mer is unusually upfield shifted (marked by *4) as observed for d(TCGA) (21).

**B-TATA 16 mer**

Figure 5A shows a portion of the NOESY spectra of B-TATA 16 mer obtained at 25°C (below the first Tm=43°C) with a mixing time of 300ms. The cross-peak connectivity between the aromatic signals and the T-CH3 is shown. The T-CH3 at 1.52 ppm has cross-peaks both with its own TH6 at 7.24 ppm and with one of the AH8 signals at 8.34 ppm. These cross-peaks are typically seen between adenines and thymines, A(5')—T(3'), leading to the assignment of this cross-peak to the thymine at position 9 (9TCH3). Another intense cross-peak is observed between the TH6 at 7.28 ppm and the T-CH3 at 1.58 ppm. This must be the thymine at position 7 (7T-CH3) and is also connected to one of the GH8 at 7.94 ppm, permitting assignment of this cross-peak to the GH8 at position 6 (6GH8). These data demonstrate that the thymine residue at position 7 in B-TATA 16 mer stacks with the guanine residue in the CGCGCG segments at position 8.

The NOESY spectra for B-TATA 16 mer was also recorded at 40°C (near the first Tm=43°C). A portion of the NOESY spectra is shown in Figure 5B. Exchange cross-peaks for each pair of the 8 and 10 AH8 are connected by the dashed lines to the diagonal
Figure 5. A selected portion of the NOESY spectra of B-TATA 16 mer recorded with a mixing time of 300ms (A) NOE cross-peaks between the T-CH$_3$ and aromatic signals at 25°C. and (B) Exchange cross-peaks for the AH8 at 40°C. The exchange networks are followed by the dashed lines.

peaks. For instance, the diagonal peak 1 at 8.46 ppm (8AH8) is connected to the peak 1' through cross-peak (a). Similarly, the diagonal peak 2 at 8.40 ppm is connected to the peak 2' through cross-peak (b). These cross-peaks correspond to the transition from the duplex to the hairpin.

Z-TATA 16 mer

a) CGCGCG Stems

Segments of the COSY and NOESY spectra of Z-TATA 16 mer are shown in Figure 6. In the COSY spectra (Figure 6A), four cross peaks, (a), (b), (c), and (d) are observed. The cross-peaks arise from scalar coupling between the CH5 and CH6. The NOESY spectra were recorded at various temperatures between 5 and 30°C. These spectra were essentially the same, with the best spectra obtained at 30°C. In the NOESY spectra (Figure 6B), a very intense cross-peak designated (e) is observed between the H1' (6.19 ppm) and the GH8 (7.78 ppm) regions. Due to (i) their characteristic chemical shifts and (ii) the observation of the cross-peak (e) at 5°C, the cross-peak (e) is not likely to arise from the chemical exchange, or either aromatic-aromatic or H1'-H1' interactions. The cross-peak (e) must, therefore, arise from the dipolar interaction between the GH8 and GH1' within the guanine residue. The NOESY spectra show the close proximity of GH8 and
Figure 6. Selected portions of (A) the COSY spectra at 30°C and (B) the NOESY spectra of Z-TATA 16 mer recorded with a mixing time of 300ms at 30°C. (A) The cross-peaks, (a), (b), (c), and (d) are due to the scalar interaction between the CH5 and the CH6. (B) The strongest cross-peak (e) indicated by an arrow is due to the interaction between the GH8 and the GH1'. The crosspeaks (a), (b), (c), and (d) are due to the scalar interaction between the CH6 and the CH5.

GH1' in space, consistent with guanine residues in the syn conformation around the β-glycoside bond, a characteristic of Z-type structures (11). The assigned signals and the chemical shifts are listed in Table II.

b) TATA Box
In contrast to the two AH8 signals in the 1-D spectra for B-TATA 16 mer (Figure 4A), several AH8 signals are observed for Z-TATA 16 mer at 5°C (Figure 4B). We have attempted to determine the conformation of the adenine residues of the TATA box by 1-D
Table II. \(^1\)H Chemical Shifts (ppm) of the Representative Signals in Z-TATA 16 mer in 10 mM phosphate buffer, 0.1M NaCl, 4M NaClO\(_4\), and D\(_2\)O, pH=6.6 at 30°C.

<table>
<thead>
<tr>
<th>Z-TATA 16 mer</th>
<th>GH8</th>
<th>CH6</th>
<th>CH5</th>
<th>T-CH(_3)</th>
<th>GH1'</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH8 8.02</td>
<td>7.78</td>
<td>7.37</td>
<td>5.79</td>
<td>1.41</td>
<td>6.19</td>
</tr>
<tr>
<td>AH8 8.09</td>
<td>7.33</td>
<td>5.71</td>
<td></td>
<td>1.46</td>
<td></td>
</tr>
<tr>
<td>AH8 8.13</td>
<td>7.41</td>
<td>5.29</td>
<td></td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>AH8 8.17</td>
<td>7.30</td>
<td>5.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AH8 8.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AH8 8.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The NOESY spectra were recorded with a mixing time of 300ms at 30°C (near the first Tm). The portion of the spectra is shown in Figure 7A. Several cross-peaks are seen between the T-CH\(_3\) and TH6 (a-c) and between the T-CH\(_3\) and AH8 (d) and GH8 (e and f). Figure 7B shows the portion of the NOESY spectra in the aromatic regions (AH8). Although the 1-D spectra of this region is crowded (Figure 4B), six well resolved cross-peaks are seen for the AH8 [marked (a) to (f) in the NOESY spectra (Figure 7B)]. Because the distance between the 8AH8 and the 10AH8 is large, these cross-peaks cannot originate from the cross-relaxation term, \(\alpha\), but probably arise from conformational exchange. Each cross-peak connects to the two diagonal peaks which, in turn, correspond to the two exchanging conformers. The exchange networks are followed and indicated by the dashed lines in Figure 7B. For instance, the diagonal peak 1 at 8.23 ppm (8AH8) is connected to the peak 1' (8.13 ppm) through cross-peak (a). Similarly, the other five cross-peaks (b-f) connect to the corresponding pairs of the diagonal peaks such as 2-2', 3-3', 4-4', 5-5', and 6-6'. Thus, for the two adenine residues, there must be six slowly exchanging conformations.

**Pre-Steady State NOE Measurements**

The cross-relaxation rates, \(\sigma\), have been measured for the CH6—CH5 of d(CG)\(_3\) and TATA 16 mer in low and high ionic strength solutions by pre-steady state NOE techniques (22,23). The initial slope of NOE enhancement, \(N_{ij}\), is related to crossrelaxation rate, \(\sigma\), and the apparent correlation time, \(\tau_{app}\), as follows (24):

\[
\text{NOE}_{ij}(t) = \sigma_{ij}t
\]

\[
\sigma_{ij} = K[J(0)−6J(2\omega)]
\]

\[
= (K/5)[\tau_{app}−6\tau_{app}]/(1+4\omega^2\tau_{app}^2)
\]

where \(K=\gamma^4h^2/10r_{ij}\), \(\omega\) is the Larmor frequency of spins, \(t\) is the irradiation time, and \(h\) is Planck's constant divided by \(2\pi\). With a fixed interproton distance, \(r_{ij}\), \(\tau_{app}\) is readily extracted.

A series of NOEs were measured at different irradiation times, \(t\), ranging from 50 to 200ms for the CH5—CH6 of B- and Z-TATA 16 mer at 5°C. Results of the NOE experiments are shown in Figure 8. A plot of the observed NOEs as a function of irradiation times, \(t\), appears in Figure 9. The initial slopes of the NOE build-up profiles yield \(\sigma=1.2\) and 0.4s\(^{-1}\), corresponding to \(\tau_{app}\) of 4.7 and 1.8ns for B- and Z-TATA 16 mer, respectively, using 2.46 A as a fixed interproton distance for the CH6—CH5 vector. Similarly, NOEs are measured for B- and Z-d(CG)\(_3\) as the reference, and \(\sigma\) are 0.7 and 0.4138.
Figure 7. Selected portion of the NOESY spectra of Z-TATA 16 mer recorded with a mixing time of 300ms at 30°C. (A) the cross-peaks between the T-CH$_3$ and the TH6 (a, b, c) and between the T-CH$_3$ and the AH8 (d) and GH8 (e and f) are shown. (B) Cross-peaks arising from conformational exchange between the AH8 are designated by (a) through (f). The exchange networks are followed by the dashed lines.

1.0s$^{-1}$, corresponding to the $\tau_{app}$ values of 2.9 and 4.0ns, respectively. These data are summarized in Table III.

DISCUSSION
Conformation of Z-TATA 16 mer
a) CGCGCG Segments
One of the questions of importance in this study is whether Z-type structures are formed in solution when one or more dA-dT base pairs are incorporated between flanking CGCGCG$_i$ segment. The secondary structure of the identical 16 mer, d[(CG)$_3$TATA(CG)$_3$], in low (0.1M phosphate buffer) and high ionic strength solutions (saturated NaCl) has been probed by $^1$H and $^{31}$P NMR spectroscopy (11). Even in saturated NaCl solution, only a small fraction of the Z-structure was detected in this molecule (11). In contrast, the NOESY spectra (Figure 6B) demonstrated that the GH8 and the GH1'
Figure 8. Pre-steady state NOE measurements at 5°C on (A) B-TATA 16 mer and (B) Z-TATA 16 mer. The $^1$H NMR spectra (reference spectra) and difference spectra (off-resonance minus on-resonance presaturation) are plotted. The presaturation pulse is applied to the internal CH6 resonances at different length of the irradiation times and the resulting NOE on the corresponding CH5 is measured. The arrow indicates the NOE observed on the CH5.

Table III. Cross-Relaxation Rates, $\sigma$, Apparent Correlation Times, $\tau_{app}$, Rotational Correlation Times, $\tau_c$, Amplitude Factor of Overall Tumbling, $\alpha$, Amplitude Factor of Internal motions, $(1-\alpha)$, Effective Correlation Times of Internal Motions, $\tau_i$, for TATA 16 mer and d(CG)$_3$ in 0.1M NaCl and 4M NaClO$_4$ at 5°C.

<table>
<thead>
<tr>
<th>DNA oligomer</th>
<th>$\sigma$ (s$^{-1}$)</th>
<th>$\tau_{app}$ (ns)</th>
<th>$\tau_c$ (ns)</th>
<th>$\alpha$</th>
<th>$(1-\alpha)$</th>
<th>$\tau_i$ (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-TATA 16 mer</td>
<td>1.2</td>
<td>4.7</td>
<td>11.4</td>
<td>0.41</td>
<td>0.59</td>
<td>0.42</td>
</tr>
<tr>
<td>Z-TATA 16 mer</td>
<td>0.4</td>
<td>1.8</td>
<td>11.8</td>
<td>0.15</td>
<td>0.85</td>
<td>0.46</td>
</tr>
<tr>
<td>B-d(CG)$_3$</td>
<td>0.7</td>
<td>2.9</td>
<td>4.3</td>
<td>0.67</td>
<td>0.33</td>
<td>0.43</td>
</tr>
<tr>
<td>Z-d(CG)$_3$</td>
<td>1.0</td>
<td>4.0</td>
<td>4.4</td>
<td>0.91</td>
<td>0.09</td>
<td>0.43</td>
</tr>
</tbody>
</table>

$^a$ Calculated from $\alpha_{obs}$. The interproton distance of 2.46 Å for the CH6—CH5 vector is used.

$^b$ Calculated from the Stokes-Einstein relation. The viscosities of the solvents were measured by a home-made capillary viscometer and are 1.52 cP for 0.1M NaCl and 2.01 cP for 4M NaClO$_4$ solutions.

$^c$ Calculated from $\alpha=\tau_{app}/\tau_c$. 

4140
are in the close proximity and that the guanine residues adopt syn conformation in 4M NaClO₄. The NOE spectra, together with the CD spectra, unambiguously establish the Z-type structure as a major component of d[(CG)₃TATA(CG)₃] in 4M NaClO₄.

b) TATA Box
In an attempt to determine if the adenine residue of Z-TATA 16 mer adopts the syn or anti conformation, we conducted a series of NOE experiments. Unfortunately, only small NOEs were observed after irradiation of the AH8 (data not shown). Plausible explanations include: (i) a larger degree of internal motion is present in the adenine residues (ωτᵥ app ≈ 1 or τᵥ app ≈ 0.6ns) relative to the CGCGCG portions (τᵥ app ≈ 1.8ns); or (ii) the adenine residues possess a unique conformation. The conformational uncertainty of the TATA box in Z-TATA 16 mer is not surprising. This is probably because the short TATA box for which it is hard to adopt the Z-form is surrounded by two well-defined Z-type CGCGCG segments, resulting in the partial unwinding of the helical structure in both the CGCGCG and the TATA regions.

Multiple Structures
The first Tm of B-TATA 16 mer (0.1M NaCl) is found at 43°C by UV melting experiments. NOESY spectra recorded at 40°C exhibit the two pairs of cross-peaks for the AH8 (Figure 5B). These data indicate that B-TATA 16 mer is interconverting with the unimolecular hairpin and are consistent with the observations for TATA 12 mer (19) and d[(CG)₂AATT(CG)₂] (25).

There are several indications that multiple conformations in Z-TATA 16 mer exist in the slow exchange regime on the NMR chemical shift difference time scale. First, the
1-D spectra show at least six AH8 and three T-CH\textsubscript{3} signals for Z-TATA 16 mer, instead of the two pairs for AH8 and T-CH\textsubscript{3} in the 1-D spectra of B-TATA 16 mer (*1 and *3, Figures 4A and 4B). Second, we have observed three, instead of two, distinctive cross-peaks between the aromatic protons and the T-CH\textsubscript{3} in the NOESY spectra of Z-TATA 16 mer (Figure 7A). Third, additional cross-peaks are seen due to chemical exchange at the lower end of the aromatic region of the Z-TATA 16 mer spectrum (Figure 7B). These data are summarized in Table IV.

In principle, the exchange rates can be obtained from the ratio of the cross-peak to diagonal-peak intensities in a 2-D exchange experiment with short mixing times (26,27). Unfortunately, in the present case diagonal peak intensities cannot be obtained with satisfactory accuracy due to the severe overlapping of the signals. Nevertheless, the chemical shift difference between the exchange sites provides the upper limit of the exchange rates \([k_{ex} < \text{difference (Hz)} \text{ in the chemical shift between the sites}].\) The chemical shift differences are about 30 Hz for most of the exchange pairs requiring exchange rates less than 30s\(^{-1}\). These data are listed in Table V, and are consistent with the reported values for TATA 12 mer (19) and d[(CG)\textsubscript{2} ATT(CG)\textsubscript{2}] (28).

The intensities of the cross-peaks (c) and (e) are much greater than the others. This is probably because magnetization transfer in the (c) and (e) conformers is more efficient than in the other conformers. We conclude that in 4M NaClO\textsubscript{4} solution the TATA box of TATA 16 mer adopts non-Z-conformation, and has multiple conformations in slow exchange regime.

**Conformational Flexibility**

The overall tumbling time, \(\tau_c\), of DNA oligomers can be readily calculated from the Stokes-Einstein relation, assuming that short DNA duplexes act as isotropic rotors. This procedure provides a reasonable approximation of the overall tumbling times for short DNA oligomers (22). \(\tau_c\) calculated from the Stokes-Einstein relation are 4.3 and 4.4ns for B- and Z-d(CG)\textsubscript{3}, respectively, at 5°C. The molecular volume estimates used were \(\pi(13)^3\times3.4.6\ A^3\) (29) for B- and \(\pi(11)^3\times3.7.6\ A^3\) (30) for Zd(CG)\textsubscript{3}. Using the dimension of \(\pi(13)^2\times3.4.16\) for B-TATA 16 mer and \(\pi(13)^2\times3.7.16\) for Z-TATA 16 mer \(\tau_c\) was calculated from the Stokes-Einstein relation which gave the lower limit of \(\tau_c\) (Table III).

The apparent correlation time (\(\tau_{app}=4.0\text{ns}\)) obtained from the observed cross-relaxation rate is similar to the rotational correlation time (\(\tau_c=4.4\text{ns}\)) for Z-d(CG)\textsubscript{3} calculated for a rigid rotor. However, \(\tau_{app}\) is smaller than \(\tau_c\) for B- and Z-TATA 16 mer and B-d(CG)\textsubscript{3} (Table III). The large difference in the magnitude between \(\tau_{app}\) and \(\tau_c\) for B- and Z-TATA 16 mer requires a significant contribution from the internal motion to the cross-relaxation rate.

Motional effects are expressed in terms of the spectral density functions, \(J(\omega)\). Assuming that only a single internal motion contributes to the cross-relaxation rate, the spectral density
Table V. Chemical Shift Differences Between Conformational Exchange Sites for the AH8 of B-TATA 16 mer at 40°C in 0.1M NaCl and Z-TATA 16 mer at 30°C in 4M NaClO4.

<table>
<thead>
<tr>
<th>Sites</th>
<th>B-TATA 16mer</th>
<th>Z-TATA 16 mer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δδ (ppm)</td>
<td>Hz</td>
</tr>
<tr>
<td>1 to 1'</td>
<td>0.093</td>
<td>28</td>
</tr>
<tr>
<td>2 to 2'</td>
<td>0.097</td>
<td>29</td>
</tr>
<tr>
<td>1 to 1'</td>
<td>0.096</td>
<td>29</td>
</tr>
<tr>
<td>2 to 2'</td>
<td>0.098</td>
<td>29</td>
</tr>
<tr>
<td>3 to 3'</td>
<td>0.100</td>
<td>30</td>
</tr>
<tr>
<td>4 to 4'</td>
<td>0.096</td>
<td>29</td>
</tr>
<tr>
<td>5 to 5'</td>
<td>0.132</td>
<td>40</td>
</tr>
<tr>
<td>6 to 6'</td>
<td>0.076</td>
<td>23</td>
</tr>
</tbody>
</table>

function, J(ω), can be given as a sum of two Lorentzians (31): one for the overall tumbling and the other for the internal motions;

\[ J(ω) = \alpha \cdot τ_c \cdot (1 + ω^2 τ_i^2)^{-1} + (1 - \alpha) \cdot τ_i \cdot (1 + ω^2 τ_i^2)^{-1} \]

where \( ω \) is the angular frequency, \( τ_c \) is the correlation time, and \( τ_i \) is the internal correlation time. The amplitude factors for the overall and the internal motions, respectively, are \( \alpha \) and \( 1 - \alpha \).

The α corresponding to the generalized order parameter, \( S^2 \), derived from the trajectories of molecular dynamics simulations and is defined by (32):

\[ S^2 = \frac{α}{τ_{app}/τ_c} = \frac{α_{obs}}{σ_c} \]

where \( S^2 \) is the observed cross-relaxation rate for a particular vector, and \( σ_c \) is the calculated cross-relaxation rate for a rigid system undergoing only isotropic tumbling. From \( τ_{app} \) and \( τ_c \) (or from \( α_{obs} \) and \( α \)), \( α \) and \( 1 - α \) may be determined. Incorporation of Eq.(3) into Eq.(2) yields \( τ_i \). While \( τ_i \) are similar in B- and Z-d(CG)3 and B- and Z-TATA 16 mer, the amplitude factor of internal motion of TATA 16 mer are larger than those of d(CG)3. These data appear in Table III. One likely motion of the CH6—CH5 vector is an out of base plane motion (20).

These results demonstrate that (i) the CGCGCG segments of TATA 16 mer possess larger amount of internal motion than the corresponding d(CG)3 regardless of the helical structure, and (ii) the CGCGCG segments of Z-TATA 16 mer exhibits larger internal motion than those of B-TATA 16 mer. The large internal motion present in Z-TATA 16 mer may arise from partial unwinding of the helical structure in the junction between the well-defined left-handed CGCGCG segments and the non Z-structure of the TATA box.

ACKNOWLEDGEMENTS

This research was supported by the Research Corporation and the American Cancer Society, Illinois Division Grant # 87-30. We wish to thank Dr. A.S. Benight for collecting UV melting data and Mr. K. Kumaralal for recording CD spectra. We thank Dr. Hare for providing his NMR data processing program. We thank Dr. G. Brubaker for careful reading and constructive criticism of this manuscript.

* To whom correspondence should be addressed.
REFERENCES