**Adenine Affects the Structure and Stability of Telomeric Sequences***

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Adenine occurs in the strand containing repeated G clusters in the telomeric DNA of a variety of organisms, including that of humans. The role of adenine has been investigated by constructing two sets of oligonucleotides each with one, two, or four copies of the telomeric sequence dTTTAGGG together with a control sequence in which T replaces the A residue, dTTTTGGG. Comparison of the stability and spectral properties of these two sequences in the presence of Na⁺ or K⁺ affords a basis for defining the role of adenine in these structures. In Na⁺, the A residue stabilizes the structure formed by each oligomer significantly, presumably by a base-pairing interaction with T. In K⁺, by contrast, there is little difference in stability. In two- and four-copy oligomers, the A sequence has a different structure from its T analog, as detected by CD spectroscopy. In the presence of either Na⁺ or K⁺, the tetrplexes of A and T interact with intercalators.

Telomeres are specialized DNA-protein structures at the ends of eukaryotic linear chromosomes, essential for chromosomal stability and the complete replication of chromosomal termini (1, 2). Telomeric DNA contains simple tandemly repeated sequences (2), with clusters of G residues in one strand (3). A conserved structural feature in telomeres is a single-stranded overhang, containing two additional copies of a G-rich sequence (12-16 nucleotides in length) in the 5' to 3' direction (2, 4, 5). Synthetic G-rich telomeric DNA oligonucleotides corresponding to the single-stranded overhang of the telomeres from a variety of organisms act as primers for telomere terminal transferase enzymes (6-11). Duplex DNA bearing these 3'-terminal overhangs shows unusual concentration dependent association in solution (12, 13). The in vitro properties of oligonucleotide models containing single or tandemly repeated telomeric sequences have been investigated in order to determine the structural and energetic basis for this interaction.

Synthetic DNA oligonucleotides with one or more copies of the G-cluster telomeric sequences form defined folded structures in solution that are stabilized by non-Watson-Crick G-G base interactions. Henderson et al. (14) made the important observation that in the presence of low concentrations of sodium or potassium ions, oligomers with four copies of a G-rich telomeric DNA sequence fold to form an intramolecular, double-stranded hairpin loop structure with rapid electrophoretic migration in nondenaturing gel electrophoresis. The N7 position of each guanine in the cluster region of G residues is inaccessible to the methylating agent dimethyl sulfate, consistent with involvement of all G residues in formation of multistrand G4-DNA structures (15). DNA oligomers corresponding to four copies of telomeric sequences from the protozoa Oxytricha (dTTTTGGGG) and Tetrahymena (dTTTGGG) form compact, intramolecularly folded G-quartet structures in Na⁺ or K⁺ (16). Formation of fold-back G-quartet structure via dimerization of hairpin-looped monomers in Na⁺ or K⁺ was suggested by Sundquist and Klug (17) when they examined two copies of telomeric sequences of *Tetrahymena*. These telomeric structural motifs may play a direct role in telomere function (3, 16, 17). For example, formation of K⁺-stabilized folded structure inhibits the action of the telomerase from *Oxytricha nova* (18). The different response of these sequences to sodium and potassium has led Sen and Gilbert (19) to postulate a sodium-potassium switch in telomeric structure that might also have functional implications. Thus, it is of interest to investigate the determinants of stability and structure in G-rich strand of telomeric sequences.

Several naturally occurring telomeric sequences identified so far contain only three contiguous guanines with an adenine residue flanking the terminal G (3). The sequences from protozoa contain four adjacent Gs instead. This raises the question of what the role of the adenine might be in the formation of telomeric structures *in vitro*. To investigate the role of this adenine, we have synthesized two series of oligonucleotides corresponding to one copy, two copies, or four copies of the telomere sequence dTTTAGGG, and its T-containing variant, dTTTTGGG, for studies of their structure and stability.

We and others have previously investigated the corresponding properties of a similar set of copies of the *Oxytricha* sequence, dTTTTGGGG, providing an additional calibration of associative properties for known natural copy sequences (20, 21). Different patterns of formation of tetrplex structure have been reported for this sequence, illustrated schematically in Fig. 1. Single-copy oligomers form a stable tetramer complex both in Na⁺ and K⁺ (15) (Fig. 1A) with the strands apparently parallel (21). Strands containing two copies of the sequence can form a hairpin in either Na⁺ or K⁺, which can dimerize to form a tetrplex structure, as sketched in Fig. 1B. The strands in this complex probably run antiparallel (17). Four copies of the sequence in a chain can fold to yield a compact intramolecular G-quartet structure, as sketched in Fig. 1C (16). These structures provide a reference for interpreting the results of mobility experiments with the A and T series of oligomers in the presence of different salts.

In this paper, we report the results of electrophoretic and chemical analysis of the A and T variants. The structures of these oligonucleotides in Na⁺ or K⁺ have been investigated...
by CD spectroscopy, chemical footprinting and UV cross-linking assays. Each series of oligonucleotides can assume intra- or intermolecular folded structures in Na⁺ or K⁺, although the finer details of the structures differ appreciably. We have further measured their thermal stability using CD melting experiments. Oligonucleotides containing A are much more stable than those with T in Na⁺; each of these is less stable than the d(T₄G₄) sequence. Finally, we demonstrate that these oligonucleotides interact with an intercalating drug in Na⁺ or K⁺ using the chemical footprinting reagent methidiumpropyl-EDTA-Fe(II) (MPE-Fe(II))

**Materials and Methods**

*Synthesis and Purification of Oligonucleotides*—Oligonucleotides used in this study were synthesized on an ABI 391 PCR-MATE EP™ DNA synthesizer, and deprotected by routine phosphoramidite procedures (23). Strands were purified by preparative high pressure liquid chromatography on a Du Pont Zorbax Bio Series oligonucleotide column, following the manufacturer’s recommended elution protocol. Oligonucleotides were labeled at their 5’-termini using T4 polynucleotide kinase (Boehringer); the labeled strands were purified by polyacrylamide gel electrophoresis.

*Solution Preparation*—The concentration of DNA strands was determined spectrophotometrically at 260 nm and 80 °C (24). The concentration of ethidium bromide was determined by absorption using an extinction coefficient of ε₂₆₀ = 5680 M⁻¹ cm⁻¹ (25). DNA solutions except for CD samples were prepared in a 10 mM Tris-HCl buffer containing 0.1 mM EDTA, 200 mM NaCl or KCl, adjusted to pH 7. The DNA strands of d(T₆G₄) and d(T₃A₃G₄) were evaporated to dryness at 50 °C over 10 h, and then dissolved in 10 mM Tris-HCl buffer (pH 7), and 0.1 mM EDTA. The DNA samples were incubated at 4 °C for 48 h. DNA solutions of d(T₆G₄)₂, d(T₃A₃G₄)₂, d(T₆G₄)₃, and d(T₃A₃G₄)₃ were prepared by heating to 95 °C for 2 min, cooling slowly, and incubating at room temperature for 24 h and at 4 °C for 12 h.

*CD Spectroscopy and CD Melting Curves*—CD spectra were recorded using an AVIV model 60DS CD spectropolarimeter equipped with a programmable, thermo electrically controlled cell holder (Aviv Associate, Lakewood, NJ). DNA solutions were prepared in a 10 mM phosphate buffer containing 0.1 mM EDTA, 200 mM NaCl or KCl, adjusted to pH 7. Each spectrum corresponds to an average of three scans from which the buffer background was subtracted. The quantity ε₁₋₂ was calculated for each species by dividing the apparent signal in degrees, θ, by 3.3C, where C is defined in moles of residues. CD melting curves were recorded at 265 and 290 nm. CD at 4 °C was normalized to 1.0 in these experiments. Samples were heated at a rate of 0.5 °C/min. These melting curves allow us to measure the transition temperatures, Tm, the midpoints of the order-disorder transition of these DNA molecules, as well as the relevant thermodynamic parameters. These parameters were calculated by using standard procedures reported by Marky and Breslauer (26) and correspond to a two-state approximation of the helix-coil transition of each molecule.

**MPE-Fe(II) Cleavage Reaction**—Our procedure followed that of van Dyke and Dervan (22) and Guo et al. (28). A volume (10 µl) of

![Fig. 1. Schematic models for proposed structures involving one, two, and four copies of G-cluster oligonucleotides. Straight or bent lines indicate free or looped T₄ (or T₃A) sequences, while the zig-zag line denotes oligo G₄. A, formation of a parallel strand tetramer from four single-copy oligomers of a sequence such as d(T₆G₄)₂, with dimerization of two hairpins to yield a tetraplex one, two, and four-copy oligomer, d(T₆G₄)₄ for example (16).](image)

![Fig. 2. Polyacrylamide gel electrophoresis of the telomeric oligonucleotides under nonadenaturing conditions. Shown is an autoradiograph of the native gels in which the telomeric oligonucleotides were subjected to gel electrophoresis in 53.4 mM Tris borate (pH 8.3), and 1 mM EDTA (0.6 X TBE) (A), 0.6 X TBE + 50 mM NaCl (B), and 0.6 X TBE + 50 mM KCl (C) at 4 °C. The DNA samples were prepared as described under “Materials and Methods.”](image)
DNA was exposed to 10 μM Fe(II) and 10 μM MPE in a buffer of 10 mM Tris·HCl (pH 7), 0.1 mM EDTA, 200 mM NaCl or KCl for 15 min at 4 °C, followed by addition of 4 mM dithiothreitol for 45 min. The reaction was stopped by extraction with 1-butanol and precipitation with ethanol.

Gel Electrophoresis—For denaturing gels, samples for electrophoresis were taken up in formamide loading buffer, heated briefly to 90 °C, cooled, then run on a denaturing polyacrylamide gel at 2000 V (~50 V/cm) and 40 °C. No dyes were added in these runs. The gel was dried immediately on a vacuum drying apparatus (Hoefer), and exposed at room temperature to x-ray film without an intensifier screen. For native gels, 20% gels were run at 4 °C for 20 h at 100 V (~8 V/cm). The electrophoresis plates were jacketed and cooled with circulating water to provide a running temperature of 4 ± 1 °C. The buffer system contained 53.4 mM Tris borate-EDTA buffer, pH 8.3, (0.6 × TBE), with 50 mM of sodium or potassium salts. No tracking dyes were added to samples in these runs. The gels were exposed to x-ray film for 1 h without an intensifying screen.

RESULTS AND DISCUSSION

Both A and T Oligonucleotide Sequences Assemble or Fold in the Presence of Na+ and K+—Electrophoretic mobilities of these oligonucleotides under native conditions are shown in Fig. 2. In the absence of added salt, each oligonucleotide appears to migrate as a monomer; chains with copies of dTTTAGGG have slightly greater mobilities than those with dTTTTGGG in each case (Fig. 2A). In the presence of Na+, both dT,G₄ and dT₄AG₃ still run as monomers. However, both the four-copy species, d(T₄G₃)₄ and d(T₄AG₃)₄, show obviously enhanced mobilities in Na⁺ (Fig. 2B), consistent with forming a more compact structure (16), presumably involving intramolecular G-quartet bonding (see Fig. 1C).

Shorter chains behave differently: whereas the single-copy strand with A runs faster than that with T, the two-copy molecule d(T₄AG₃)₂ migrates more slowly than d(T₄G₃)₂ in the presence of sodium (Fig. 2B). The mobility of the latter shifts only slightly relative to an 11-mer duplex marker, while the A sequence shifts considerably. One possibility is that this is due to dimerization of the A species again to form an intermolecular tetraplex structure (see Fig. 1B).

Potassium ions have a profound effect on the stability and structure of G-cluster sequences (29). In the presence of K⁺, the single copy and double copies of dT₄G₃ and dT₄AG₃ show a large decrease in mobility. The four-copy species do not
The overall conformation of G-cluster oligonucleotides probably form tetraplex structures in solution, which are less stable and dissociate at the concentrations used in the gel experiment.

Methylation Protection Shows That All Gs in the A and T Oligonucleotides Participate in Formation of Ordered Structures in the Presence of Na+ or K+—It has been shown that the Hoogsteen G·G base pairing in tetraplex structure confers protection from methylation of the guanines by dimethyl sulfate, which preferentially methylates N7 of guanine (15, 16, 19). When oligonucleotides with one, two, and four copies of dT,G3 or dT,AGG3 are methylated by dimethyl sulfate in the TE buffer (pH 7) containing 200 mM NaCl or KCl at 4 °C, protection of N7 in all guanines of these sequences results (Fig. 3). This suggests that these telomeric oligonucleotides are structured in the presence of 200 mM NaCl or KCl. Although dT,G3, dT,AGG3, and dT,GG3 have mobilities close to those of monomers on native gel electrophoresis under conditions of 200 mM NaCl at 4 °C, these oligonucleotides probably form tetraplex structures in solution, which are less stable and dissociate at the concentrations used in the gel experiment.

A and T Oligonucleotides Show Structural Differences—CD spectroscopy provides a sensitive differential probe of the overall conformation of G-cluster oligonucleotides (20, 21, 31–33). The CD spectra of oligonucleotides, corresponding to one, two, and four copies of dT,G3 and dT,AGG3 were measured in Na+ or K+ as a function of temperature. The spectra of dT,G3 and dT,AGG3 in K+ are very similar to that of dT,G4, with a trough at 240 nm, and a peak at 265 nm for which Δε increases on heating up to about 40 °C (data not shown) (21). This is consistent with formation of a tetramer in each case, as sketched in Fig. 1A. Since the effect of substitution of adenine for thymine has only a minor effect on the absorbance spectrum, the fact that the CD spectra for dT,G3 differ in shape (e.g., maximum at 280 nm) from that of dT,AGG3 in Na+ (minimum at 280 nm) implies a structural difference between the two molecules (data not shown) and between these and dT,G4 (20). The CD profiles indicate that the overall conformation of the two-copy sequence d(T,G)2 in both Na+ and K+ differs from that of d(T,AGG)2 (Fig. 4, A and B). In Na+, d(T,G)2 has a minimum at 240 nm, and d(T,AGG)2 at 262 nm; in K+, the former has a very broad peak centered at 280 nm, while the latter has a maximum at 265 nm. By contrast, the four-copy oligomers d(T,G)4 and d(T,AGG)4 in Na+ have similar CD spectra, resembling that of d(T,G)4 (20), with a minimum at 260 nm and a positive band at 290 nm (data not shown). In K+ they have distinct CD profiles consistent with a difference in layer structure (data not shown) (20). While both the oligomers containing two and four copies of dT,G3 and dT,AGG3 would be expected to assume inter- or intramolecular tetraplex structures, a clear difference in their CD spectra is observed. Thus the adenine in the Arabidopsis sequence affects the overall conformation dramatically in oligomers containing two copies of the G-rich sequence compared to their dT,G4 counterparts (Fig. 4). The most reasonable interpretation is that the A can pair with a neighboring
Fig. 5. **UV cross-linking of the A and T oligonucleotides.** A, an autoradiograph of the 14% denaturing polyacrylamide gel is shown of the cross-linked products upon irradiation at 254 nm at 4 °C. The DNA samples were prepared in TE (lane TE) or TE with 200 mM NaCl (lane Na⁺) or KCl (lane K⁺). B, an autoradiograph of the denaturing polyacrylamide gel of the piperidine cleavage products in cross-linked species. Lane DMS contains a dimethyl sulfate ladder of unirradiated material for a marker. Lanes U and X contain cleavage products of irradiated but uncross-linked material, and cross-linked material, respectively. The cross-linked positions in lane X are indicated by arrows.
Role of A in Telomeric Repeats

Table I

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Tm°C</th>
<th>ΔG° kJ/mol</th>
<th>ΔH° kJ/mol</th>
<th>ΔS° J/(mol K)</th>
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<td>dT₄G₃</td>
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<td>58.0</td>
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<td>-271 ± 14.1</td>
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<tr>
<td>dT₄AG₃</td>
<td>K⁺</td>
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<td>-47.0 ± 2.4</td>
<td>-289 ± 14.0</td>
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<td>dT₃AG₃</td>
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<td>-6.0 ± 0.3</td>
<td>-49.4 ± 2.9</td>
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<tr>
<td>dT₃AG₃</td>
<td>K⁺</td>
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<td>-9.9 ± 0.5</td>
<td>-49.3 ± 2.6</td>
</tr>
<tr>
<td>dT₄AG₃</td>
<td>Na⁺</td>
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<td>-7.8 ± 0.4</td>
<td>-42.4 ± 2.2</td>
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<tr>
<td>dT₄AG₃</td>
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<td>62.5</td>
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<td>-81.1 ± 4.1</td>
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<tr>
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<td>-1.3 ± 0.1</td>
<td>-34.8 ± 1.5</td>
</tr>
<tr>
<td>dT₄AG₃</td>
<td>Na⁺</td>
<td>57.5</td>
<td>-3.8 ± 0.2</td>
<td>-39.3 ± 2.0</td>
</tr>
<tr>
<td>dT₄AG₃</td>
<td>Na⁺</td>
<td>45.5</td>
<td>-1.9 ± 0.1</td>
<td>-30.1 ± 1.6</td>
</tr>
<tr>
<td>dT₄AG₃</td>
<td>K⁺</td>
<td>57.1</td>
<td>-3.1 ± 0.2</td>
<td>-32.1 ± 1.7</td>
</tr>
</tbody>
</table>

*All melting experiments were done in a 10 mM phosphate buffer (pH 7), 0.1 mM EDTA, 200 mM NaCl or KCl.

Calculated for 10⁻⁴ M single-strand concentration.

Calculated at 25°C assuming ΔC_p = 0.

Calculated from 1/T_m versus ln C_p parameters with the molecularity n = 4.

Calculated from 1/T_m versus ln C_p parameters with n = 2.

Calculated from curve fit parameters with n = 1.

T in certain circumstances, but not in others.

**UV-induced Cross-linking Assay Shows Formation of G Tetraplex Structures in Na⁺ or K⁺**—UV-induced cross-linking is a very useful probe of the folded structure in telomeric sequences (16, 21). Here, we have used UV cross-linking to study the structure of oligonucleotides with one, two, and four copies of dT₄G₃ and dT₄AG₃ in Na⁺ and K⁺. Fig. 5A shows the results of these cross-linking experiments. In the presence of K⁺, both dT₄G₃ and dT₄AG₃ generate cross-linked products including dimers, trimers, and tetramers, indicating that both species form a four-stranded parallel tetraplex structure (21). Only dimers can be detected in the presence of Na⁺ (Fig. 5A). By contrast, d(T₄G₃)₂ and d(T₄AG₃)₂ dimers are observed following irradiation in either Na⁺ or K⁺. Both d(T₄G₃)₂ and d(T₄AG₃)₂ form cross-linked species with enhanced mobility on denaturing polyacrylamide gels, consistent with the observation by Williamson et al. (16). We attribute this to the formation of intramolecular structure in Na⁺ or K⁺ that leads to proximity of two of the T-containing loops (Fig. 1C). To identify the positions of cross-linking, we preferentially cleaved the cross-linked nucleotides in the cross-linked species with piperidine (16). The labeled oligonucleotides were annealed in Na⁺ or K⁺, and irradiated at 254 nm. The cross-linked species and un-cross-linked controls were gel purified, following which the labeled DNAs were treated with piperidine. The sequence ladders from a dimethyl sulfate reaction are also shown for reference. The piperidine-induced strand scission experiments are shown in Fig. 5B, and the positions of cross-linking are indicated by the arrows. The results show that the complexes formed from the A or T-containing sequences are both metal and sequence dependent, consistent with the CD data. For example, d(T₄AG₃)₄ in Na⁺ is preferentially cross-linked at position G5, while the corresponding position in K⁺ only has a very light band (Fig. 5B); on the other hand d(T₄AG₃)₂ in both Na⁺ and K⁺ is strongly cross-linked at position A4, while no cross-linking is detected in its T counterpart (Fig. 5B). Furthermore, the UV cross-linking data also suggest that more than one conformation of these A and T oligonucleotides exists in solution, as detected by the presence of several cross-linked sites in each molecule (Fig. 5B).

**Thermodynamic Profiles for Complex Formation by A- and**
Fig. 7. MPE·Fe(II) cleavage of the A and T oligonucleotides. An autoradiograph of the denaturing gels is shown of the MPE·e(II) cleavage products. The DNA samples were prepared in 10 mM Tris·HCl (pH 7), 0.1 mM EDTA, with 200 mM NaCl (lane Na⁺) or KCl (lane K⁺). Lane U is an uncut control. Sites of strong reactivity are indicated by arrows.

*T-containing G Clusters*—The relative energetics of the interactions that stabilize these complexes, and how these might depend on sequence, are of interest. We have evaluated thermodynamics of forming tetraplex structures from oligonucleotides corresponding to the *Arabidopsis* telomeric sequence in Na⁺ or K⁺ in two different ways. First, the midpoint thermal denaturation temperatures of these DNA molecules were determined from the CD melting curves as a function of total DNA strand concentration. Second, the van't Hoff enthalpies were estimated by fitting the shapes of the transition profiles. The van't Hoff enthalpy (δH), entropy (δS), and free energy (δG) were calculated using standard procedures (26), effectively applying a two-state approximation to the helix-coil transition for each molecule. Apart from those of dT₄G₃ and dT₃AG₃ in Na⁺, the CD melting profiles are monophasic. Fig. 6A shows normalized melting curves for d(T₄G₃) in K⁺, and d(T₄G₃) in Na⁺ or K⁺, which are independent of strand concentration as expected for intramolecular structures (data not shown). Plots of 1/Tₘ versus ln Cₚ for dT₄G₃ and dT₃AG₃ in Na⁺ in K⁺, and d(T₄G₃) in Na⁺ or K⁺ are shown in Fig. 6B and C. Thermodynamic parameters derived from fits of melting curves or In Cₚ plots are summarized in Table I. The results show that oligonucleotides composed of dT₄G₃ are substantially more stable than those corresponding to...
dT,G: in Na', while in each case there are only minor differences in the presence of K'.

**Site-specific Interaction of the A and T Oligonucleotides with an Intercalating Drug**—We have shown that intercalators such as ethidium interact with DNA containing guanine clusters that have a four-layer, but not a two-layer, tetraplex structure, via an intercalative mechanism (20). Since the dimethyl sulfate protection assay suggests that all the folded structures formed from the A and T oligonucleotides contain three layers of four hydrogen bonded guanine bases (G4 layers) in Na' or K' (Fig. 3), we would like to compare the drug-interacting potential of these molecules. Accordingly, we used a chemical footprinting technique to locate potential drug-binding site(s) in these G-rich telomeric oligomers (20). Dervan's chemical reagent MPE-Fe(II) can be a very useful probe in our present inquiry since it consists of an ethidium-like ring system linked to a reactive EDTA-Fe(II) group that can initiate DNA strand cleavage in the vicinity of the binding site(s) (34). As can be seen in Fig. 7, strong reactivity at G7 in both dT,G, and dT,AG, is detected in Na' or K' (20). In the case of the two-copy molecules, dT,G, and d(T,AG),, enhanced scission activity was identified in the G-rich region: G6, G7, G12-14, with slightly weaker cleavage at positions G12-14, and G19-21, and weak one at the loop regions (Fig. 7). The cleavage pattern produced by MPE-Fe(II) is consistent with specific drug binding site at the G region of these oligonucleotides in Na' or K'. Interestingly, the results also suggest that T-strings in the folded structures are sites of drug binding. The results presented here demonstrate that DNA complexes with three layer tetraplex base pairing can interact with intercalating drugs, as well as the four layer complex we have reported on previously (20). In the MPE-Fe(II) experiment, the preferred site could be eliminated by competition with a second nonreactive intercalator, ethidium (20). This suggests that the binding site or sites involved might be favorable for intercalative binding agents in general.

**CONCLUSION**

The known sequence patterns in telomeric repeats from protozoa and higher eucaryotes contain G clusters, T clusters, and sometimes A as well (2). (Yeasts contain more unusual patterns, and we have not attempted to consider these in this work.) It is of interest to try to define the differential structure and stability of such sequences in forming inter- and intramolecular assemblies in vitro. Here we have focused on the role of an A versus T substitution in the sequence dT,AG, identified in the telomeric DNA of the plant Arabidopsis (35). So far as we know, the T version of this sequence, dT,G, has not been identified as a telomeric repeat. What we find is that the single A versus T difference leads to changes in the stability and the structure of molecules containing two or more copies of these sequences that are not easily predictable. One can conclude from Table I that in the presence of K', complexes of any A and T sequences have similar stabilities. They do not have similar structures, as seen from the CD spectra. By contrast, in Na', the stability and structure of the sequences with A differ from those that lack A; the A residue appears to stabilize structures such as are shown in Fig. 1, B and C. It seems reasonable to attribute this to base pairing involving a T rather than to stacking of the A on an adjacent G. The four-copy versions of these sequences behave similarly to each other, and form intramolecular folded back structures (Fig. 1C) as does the sequence dT,G, from the protozoan, Oxytricha. In Na', the structure formed from the A sequence is more stable than that containing only T, whereas the K' structures are identical in stability. Thus the structure in Na' differs from that in K', perhaps allowing A-T pairing that is disrupted in the latter. We have suggested for example that K' allows partial formation of four-layer tetraplex structure in the four-copy oligomer dT,(G), (21). A stabilizes the formation of tetraplex structure in the case of each one-copy oligomer, although the A sequence is much less stable than the dT,G, sequence we have studied previously (21). Each single-copy tetramer complex (Fig. 1A) stabilized by Na' or K' has parallel strands and is capable of tightly binding an intercalative dye such as ethidium (20). The two-copy molecules differ in structure and in stability from the other motifs. Since two copies may correspond to the length of a single telomeric repeat (20), the effect of sequence in a telomeric repeat (how the region responds to physical or chemical damage for example) is thus modulated by both context and by the environment, especially K' concentration. An additional point that emerges from these results is that the stability of the structure formed from sequences with three adjacent Gs is much lower (with or without the A) than that of analogous sequences containing four adjacent Gs (20, 21). Thus the assembly process is highly cooperative: in principle, additional Gs should further stabilize the association between single-copy sequences or two copy molecules but not the structure formed from four-copy versions.

**REFERENCES**