Influence of loop size on the stability of intramolecular DNA quadruplexes

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ABSTRACT

We have determined the stability of intramolecular DNA quadruplexes in which the four G₃-tracts are connected by non-nucleosidic linkers containing propanediol, octanediol or hexaethylene glycol, replacing the TTA loops in the human telomeric repeat sequence. We find that these sequences all fold to form intramolecular complexes, which are stabilized by lithium < sodium < potassium. Quadruplex stability increases in the order propanediol < hexaethylene glycol < octanediol. The shallower shape of the melting profile with propanediol linkers and its lower dependency on potassium concentration suggests that this complex contains fewer stacks of G-quartets. The sequence with octanediol linkers displays a biphasic melting profile, suggesting that it can adopt more than one stable structure. All these complexes display melting temperatures above 310 K in the presence of 10 mM lithium, without added potassium, in contrast to the telomeric repeat sequence. These complexes also fold much faster than the telomeric repeat and there is little or no hysteresis between their melting and annealing profiles. In contrast, the human telomeric repeat sequence and a complex containing non-nucleosidic linkers show positive maxima at 264 nm, with negative minima ~244 nm, which are characteristic of parallel quadruplex structures. These results show that the structure and stability of intramolecular quadruplexes is profoundly influenced by the length and composition of the loops.

INTRODUCTION

Guanine-rich nucleic acid sequences can associate into four-stranded structures that contain stacks of G-quartets (1–4). (Fig. 1A). Intermolecular complexes are generated by the association of four separate DNA molecules (5–7) or by dimerization of molecules that contain two G-tracts (8,9), while intramolecular quadruplexes (as shown in Fig. 1B) are formed by folding a single strand of DNA (10–14). The formation of these structures requires the presence of monovalent cations (especially potassium) (15–17) while stable folding is inhibited by lithium (18). The cations fit within the central core of guanine carboxyls and can lie between or within the plane of each quartet.

G-rich sequences with the ability to form quadruplex structures are found in telomeric regions of DNA, which contain highly repeated sequences such as (GGGTAA)ₙ in humans and most other higher eukaryotes (19–21), (GGG-GTTT)ₙ in Tetrahymena and (GGGGTHTT)ₙ in Oxytricha and Stylochla (22). Similar sequences are also found in the promoters of several genes including c-myc (23,24), the immunoglobulin switch region (16), insulin regulatory sequences (25) and in fragile X syndrome (26). Quadruplex formation may also be involved in the dimerization of HIV RNA (27), and this structure is found in the core of a number of aptamers including the thrombin binding aptamer (11,28,29) and an inhibitor of HIV integrase (30–32). Quadruplex-forming oligonucleotides have also been suggested as potassium sensing agents (33), and as nanowires for molecular nanoelectronics (34).

High resolution X-ray (7,13,35) and NMR (9,11,12,14) structures have been determined for several DNA quadruplexes and reveal that these complexes are polymorphic. Intermolecular quadruplexes adopt a structure in which all the strands are parallel, while the arrangement in intramolecular complexes can be parallel, antiparallel or a combination of both (3). In the usual representation of the intramolecular quadruplex (Fig. 1B) the strands are antiparallel, with the TTA loops (crossed or uncrossed) at the top and bottom of the stack. However, a crystal structure of the potassium complex has revealed that this sequence can also adopt a fully parallel structure in which the loops run between the bottom of one stack and the top of the other (13). The structure may also be affected by the nature of the monovalent cation.

Intramolecular quadruplexes form stable structures, especially in the presence of potassium, though small changes in the sequence can have large effects on the structure and stability (36). The human (GGGTAA)₄ and Oxytricha (GGGGTTT)₄ sequences fold to form stacks of three and

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four G-quartets respectively (12–14). However, the Tetrahymena sequence (GGGGTT)₄ only forms three stacked quartets, which are linked by variable length loops containing TGGT, TTG and TT (37). In contrast the HIV integrase inhibitor (GGGT)₄ adopts an extremely stable structure that contains only two stacked quartets, linked by GT in the loops (30–32). The bases in the loops can also stack against the terminal quartets. Intermolecular quadruplexes are known to form extremely slowly (38) and are therefore not suitable for investigation by melting experiments. In contrast, intramolecular complexes appear to fold much faster and generally do not show hysteresis between their melting and annealing curves (29). However, folding of the HIV integrase inhibitor in the presence of potassium includes a slow step, which is thought to involve a structural rearrangement (30). Other intramolecular quadruplexes have also been shown to display different melting and annealing profiles, especially in the presence of low concentrations of potassium (39).

There have been few studies that examine the effect of loops on quadruplex stability and most of these have changed the base composition and length (29,39). In the present study we have examined the properties of oligonucleotides in which the four G₃-tracks are separated by non-nucleosidic linkers of different lengths. For this we have used the linkers shown in Figure 1C, containing propanediol, octanediol and hexaethylene glycol. The stability of these intramolecular complexes was determined using oligonucleotides that contain a fluorophore and quencher at one end and a quencher (methyl red) at the other end (39,40). When the oligonucleotide folds into an intramolecular complex these groups are in close proximity and the fluorescence is quenched. When the structure melts these groups become separated and there is a large increase in the fluorescence signal. This technique depends on collisional (proximal) quenching between the fluorophore and quencher and is similar to other studies which have measured the fluorescence energy transfer between fluorescein and tetramethylrhodamine (41,42). The structures adopted by these sequences have been probed by circular dichroism (CD), since parallel and antiparallel arrangements of the strands usually generate characteristic CD spectra (43–46).

MATERIALS AND METHODS

Oligonucleotides

Oligonucleotides containing fluorophores and quenchers were synthesized on an Applied Biosystems 394 DNA/RNA synthesizer on either the 0.2 or 1 μmol scale and were purchased from Oswel DNA service (Southampton). These were purified by HPLC or on denaturing polyacrylamide gels. Methyl red and fluorescein were incorporated into the oligonucleotides using MeRed-dR or Fam-dR (40). The sequences of the oligonucleotides used in these studies are shown in Figure 1C.

Fluorescence melting

Fluorescence melting curves were determined in a Roche LightCycler, as previously described (40) in a total reaction volume of 20 μl. For each reaction the final oligonucleotide
concentration was 0.25 μM, diluted in 10 mM lithium phosphate pH 7.4, supplemented with various concentrations of NaCl or KCl. The LightCycler has one excitation source (488 nm) and the changes in fluorescence were measured at 520 nm. In a typical experiment the oligonucleotides were first denatured by heating to 95°C for 5 min. They were then annealed by cooling to 30°C at a rate of 0.1°C s⁻¹ (the slowest heating and cooling rate for the LightCycler). The samples were held at 30°C before heating to 95°C at 0.1°C s⁻¹. Recordings were taken during both the annealing and melting steps. It was noted that for several oligonucleotides, as described in the Results, there was significant hysteresis between the annealing and melting curves, indicating the melting profile was not at thermodynamic equilibrium. Melting experiments were therefore performed at a slower rate of heating and cooling, by increasing the temperature in 1°C steps, leaving the samples to equilibrate for 5 min between each reading. No hysteresis was observed at this much slower rate of temperature change (0.2°C min⁻¹).

**Data analysis**

Tm values were obtained from the minima in the first derivatives of the melting profiles using the LightCycler software or, together with ΔH, from van’t Hoff analysis of the melting profiles using FigP for Windows. This analysis assumes a simple two-state equilibrium between the folded and unfolded forms. In some instances the melting curves showed a linear change in fluorescence with temperature in regions outside the melting transition. This was accounted for by fitting a straight line to the first and last portions of the fluorescence curve. All reactions were performed at least twice and the calculated Tm values usually differed by < 0.5 K with a 5% variation in ΔH. Since ΔG = 0 at the Tm, ΔS was estimated as ΔH/Tm. Values for ΔG at 310 K were then estimated by assuming that ΔCP = 0. In some instances n, the number of specifically bound monovalent cations, was calculated from the slopes of plots of ΔG against log[|M⁺|] as previously described (30,47).

This analysis assumes that the oligonucleotides form folded, intramolecular, complexes and that the strands do not associate to generate intermolecular structures. This was tested for each oligonucleotide by examining the concentration dependence of the melting profiles, comparing the Tm values between 0.15 and 1.5 μM. As expected this revealed no significant changes in Tm, consistent with the formation of intramolecular complexes.

**Circular dichroism**

CD spectra of the oligonucleotides were measured on a Jasco J-720 spectropolarimeter. The fluorescently labelled oligonucleotides were used at a concentration of 5 μM and were dissolved in 10 mM lithium phosphate pH 7.4 containing 50 mM sodium or potassium chloride. These samples were boiled for 3 min before slowly cooling to room temperature and incubating overnight. Spectra were collected between 320–200 nm, using 16 scans at 100 nm min⁻¹, 1 s response time, 1 nm bandwidth. A buffer baseline was subtracted from each sample spectrum and the final spectra were normalized to have zero ellipticity at 320 nm.

**RESULTS**

**Fluorescence melting profiles**

The sequences of the oligonucleotides used in this work are shown in Figure 1. In these sequences several different non-nucleosidic linkers replace the TTA linker in the human telomeric repeat. In the telomeric sequence, C₃’ of the terminal G in each tract is separated from C₄’ of the first G in the next tract by 23 bonds and four phosphate groups. Replacing this with propanediol, octanediol or hexaethylene glycol changes the distance to 11, 16 and 25 bonds respectively and utilizes only two phosphates. The use of two hexaethylene glycol linkers increases the length to 45 bonds with three phosphates. These oligonucleotides enable us to explore how loop length and charge affect quadruplex stability.

Typical fluorescence melting curves for the five oligonucleotides, in the presence and absence of sodium, are shown in Figure 2. In these experiments the buffer used was 10 mM lithium phosphate, as this cation is known to destabilize quadruplexes, and various concentrations of sodium chloride were added to this. The temperature was changed at a rate of 0.1°C s⁻¹. All the oligonucleotides show temperature-dependent changes in fluorescence, which vary with sodium concentration. It can be seen that the least stable complexes are generated with GGG-H2 and the human telomeric repeat.
GGG-TTA. As expected these oligonucleotides have a high fluorescence in the presence of lithium alone, suggesting that they do not adopt a folded structure under these conditions. On addition of sodium chloride the melting curves typical of quadruplex formation are produced, from which the Tm values presented in Table 1 were estimated. The stabilization by sodium is not simply due to the increase in ionic strength, as addition of an equivalent concentration of lithium chloride has no effect. The curves in the presence of 40 mM NaCl show no hysteresis between the melting and annealing profiles, suggesting that the reactions are at thermodynamic equilibrium and that there are no slow steps in either the association or dissociation reactions. GGG-TTA shows some hysteresis at lower sodium concentrations, in which the Tm for melting is higher than for annealing. Under these conditions the quadruplex formed with GGG-H2 is too unstable to measure. It can be seen that GGG-H1 adopts a much more stable structure than GGG-H2, and a clear melting profile is obtained with this oligonucleotide, even in the absence of added sodium. Once again there is no hysteresis, except at the lowest concentrations of sodium. GGG-Oct and GGG-Prop also produce melting profiles in the absence of sodium, though these have different shapes. The profiles with GGG-Prop, which has the shortest linker, are much shallower, as evidenced by the low value for ΔH, and show a smaller dependence on sodium ion concentration. The melting profiles with GGG-Oct have an unusual shape. Although this oligonucleotide shows a clear melting transition in the absence of sodium, suggesting that it has adopted a folded structure, the initial part shows a linear increase in fluorescence with temperature. The effect is even more pronounced on addition of sodium. This clearly cannot represent a simple two-state equilibrium, suggesting that this sequence adopts multiple structural forms. We were therefore unable to obtain ΔH values for this transition from a simple van’t Hoff analysis.

Similar melting experiments were performed with these oligonucleotides in the presence of potassium chloride. Representative melting and annealing curves for these are shown in Figure 3 and the Tm values are summarized in Table 2. It can be seen that, under these conditions, several of the oligonucleotides show hysteresis between the heating and cooling curves. The curves for GGG-Oct and GGG-Prop are

<table>
<thead>
<tr>
<th>Table 1. Tm values for melting and annealing of the five oligonucleotides, determined in 10 mM lithium phosphate pH 7.4, containing different concentrations of sodium chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>GGG-Prop</td>
</tr>
<tr>
<td>Melt</td>
</tr>
<tr>
<td>Anneal</td>
</tr>
<tr>
<td>GGG-Oct</td>
</tr>
<tr>
<td>Anneal</td>
</tr>
<tr>
<td>GGG-H1</td>
</tr>
<tr>
<td>Anneal</td>
</tr>
<tr>
<td>GGG-H2</td>
</tr>
<tr>
<td>Melt</td>
</tr>
<tr>
<td>Anneal</td>
</tr>
<tr>
<td>GGG-HT</td>
</tr>
<tr>
<td>Anneal</td>
</tr>
</tbody>
</table>

aIndicates a very broad transition.

bΔH values (kJ mol⁻¹) determined from van’t Hoff analysis of the melting profiles in the presence of 40 mM sodium chloride. The samples were heated and cooled at a rate of 0.1°C s⁻¹.
fully reversible, while the melting $T_m$ for GGG-H1 are $\approx 3-4$ K higher than the annealing curves. In contrast GGG-H2 shows considerable hysteresis, and the difference between the heating and annealing curves is greatest at low potassium concentration. In the presence of 5 mM KCl the apparent melting $T_m$ is 339 K while the $T_m$ for the annealing reaction is too low to measure (i.e. <310 K), implying a difference of at least 30 K. The annealing reactions can be measured at higher potassium concentrations, and the $\Delta T_m$ between melting and annealing is 27 and 11 K at 10 and 40 mM KCl respectively. It is clear that, at this rate of heating (0.1°C s$^{-1}$), the melting curves are not at thermodynamic equilibrium and the folding reaction must include at least one slow step. A similar, though less pronounced, hysteresis is evident with the human telomere sequence GGG-TTA, with a $\Delta T_m$ of 4 K in the presence of 40 mM KCl. In this case the melting (but not annealing) curves are an unusual biphasic shape at intermediate potassium concentrations (this is most clearly evident in Fig. 3 for the melting curve in the presence of 5 mM KCl) and two melting transitions are reported in Table 2. The relative amplitudes of the two components vary with the potassium concentration; the lower $T_m$ is more abundant at low concentrations, while the higher $T_m$ dominates at high potassium. The lowest of these $T_m$ values is similar to the annealing $T_m$.

It is clear from these results that this rate of temperature change is too fast to permit a proper analysis of the melting profiles. The experiments were therefore repeated at a much slower rate of heating and cooling (0.2°C min$^{-1}$). Since the slowest rate of heating for the LightCycler is 0.1°C s$^{-1}$ we achieved this by increasing the temperature in 1°C steps, leaving the reaction to equilibrate for 5 min before measuring the fluorescence. This produces melting and annealing profiles with fewer data points and which are therefore only accurate to within 1°C. The results of these experiments are shown in Figure 4 and the $T_m$ values are summarized in Table 3. Examination of this table reveals that there is almost no hysteresis between the melting and annealing profiles for all the oligonucleotides, except GGG-H2 in the presence of low potassium concentrations, which shows $\Delta T_m$ values of 6, 5 and 2 K in the presence of 1, 2 and 5 mM KCl respectively. This residual hysteresis is illustrated by comparing the melting and annealing profiles in the bottom right panel of Figure 4. It can be seen that these two curves also have different shapes, and this is emphasized by the very different $\Delta H$ and $\Delta G$ values estimated by van’t Hoff analysis of the melting and annealing profiles. It is clear that the kinetics of folding and unfolding of this intramolecular quadruplex are extremely slow in the

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### Table 2. $T_m$ values for melting and annealing of the five oligonucleotides, determined in 10 mM lithium phosphate pH 7.4, containing different concentrations of potassium chloride

<table>
<thead>
<tr>
<th>KCl (mM)</th>
<th>GGG-TTA</th>
<th>GGG-H2</th>
<th>GGG-H1</th>
<th>GGG-Prop</th>
<th>GGG-Oct</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>Melt</td>
<td>342.2</td>
<td>346.7</td>
<td>Too high</td>
<td>347.0</td>
</tr>
<tr>
<td></td>
<td>Anneal</td>
<td>338.0</td>
<td>335.9</td>
<td>Too high</td>
<td>346.0</td>
</tr>
<tr>
<td>10</td>
<td>Melt</td>
<td>324.0</td>
<td>345.0</td>
<td>347.4</td>
<td>335.1</td>
</tr>
<tr>
<td></td>
<td>Anneal</td>
<td>320.4</td>
<td>Too low</td>
<td>343.1</td>
<td>334.9</td>
</tr>
<tr>
<td>5</td>
<td>Melt</td>
<td>319.4</td>
<td>339.4</td>
<td>Too high</td>
<td>358.8</td>
</tr>
<tr>
<td></td>
<td>Anneal</td>
<td>320.4</td>
<td>Too low</td>
<td>343.1</td>
<td>334.9</td>
</tr>
<tr>
<td>2</td>
<td>Melt</td>
<td>314.0</td>
<td>334.5</td>
<td>341.2</td>
<td>328.8</td>
</tr>
<tr>
<td></td>
<td>Anneal</td>
<td>314.3</td>
<td>Too low</td>
<td>336.7</td>
<td>328.0</td>
</tr>
<tr>
<td>1</td>
<td>Melt</td>
<td>309.0</td>
<td>329.4</td>
<td>353.6</td>
<td>324.2</td>
</tr>
<tr>
<td></td>
<td>Anneal</td>
<td>309.0</td>
<td>Too low</td>
<td>332.3</td>
<td>323.5</td>
</tr>
<tr>
<td>10 mM Li</td>
<td>Melt</td>
<td>Too low</td>
<td>Too low</td>
<td>312.1</td>
<td>311.4</td>
</tr>
<tr>
<td></td>
<td>Anneal</td>
<td>Too low</td>
<td>Too low</td>
<td>309.5</td>
<td>309.8</td>
</tr>
</tbody>
</table>

*Indicates a very broad transition.

The samples were heated and cooled at a rate of 0.1°C s$^{-1}$.

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The samples were heated and cooled at a rate of 0.2 K min⁻¹. The first value for Tₘ was determined from van’t Hoff analysis of the melting profiles, while the second (in italics) was estimated from the maximum in the first derivative of the melting profile, using the LightCycler programme.

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>ΔH (kJ mol⁻¹)</th>
<th>Tₘ (K)</th>
<th>ΔG (kJ mol⁻¹ at 300 K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGG-TTA</td>
<td>342/341</td>
<td>31.8</td>
<td>276/343/342</td>
</tr>
<tr>
<td>GGG-H1</td>
<td>327/326</td>
<td>17.8</td>
<td>238/326/325</td>
</tr>
<tr>
<td>GGG-H2</td>
<td>323/320</td>
<td>12.9</td>
<td>243/320/319</td>
</tr>
<tr>
<td>GGG-Prop</td>
<td>328/327</td>
<td>12.1</td>
<td>168/315/314</td>
</tr>
<tr>
<td>GGG-Oct</td>
<td>330/329</td>
<td>7.2</td>
<td>146/308/309</td>
</tr>
</tbody>
</table>

The ΔH values for these transitions show a strong dependence on the ionic strength. This is an unusual observation since simple polyelectrolyte theory suggests that there should be no change in enthalpy with salt concentration. However, this has been observed in other studies with quadruplexes and is consistent with the presence of specific ion binding sites (30). The slopes of plots of ΔG against log[M⁺] can be used to determine the stoichiometry of ion binding as previously described (30,47). These yield values of n (the number of specifically bound K⁺) of 2.6 ± 0.1 for GGG-TTA, 2.5 ± 0.4 for GGG-H1 and 3.6 ± 0.3 and 2.5 ± 0.2 for GGG-H2, derived from the annealing and melting profiles respectively. These compare favourably to values ~3, which were determined for T30695 and related intramolecular quadruplexes (30). Similar plots for the data with GGG-Prop give a value of 0.42 ± 0.04, suggesting that this oligonucleotide folds into a structure that contains fewer potassium ions, and presumably fewer G-quartets. A similar analysis is not possible for GGG-Oct as it produces melting profiles with an unusual shape.

**CD spectroscopy**

The melting profiles demonstrate that these oligonucleotides adopt stable complexes that are characteristic of quadruplex formation. These appear to be intramolecular and not intermolecular complexes, as the melting profiles are independent of oligonucleotide concentration (between 0.15 and 1.5 μM) and they run as a single band on polyacrylamide gels. However, these do not provide information on the structure of the complexes, in particular whether the strands are arranged in a parallel or antiparallel orientation. We attempted to address this question by measuring their CD spectra. Parallel quadruplexes, generated by the association of four G-rich strands, are characterized by a positive ellipticity maximum at 264 nm and a negative minimum at 240 nm, while antiparallel (intramolecular) complexes usually possess a positive maximum at 295 nm and a negative minimum at 265 nm (43–46). CD spectra for these oligonucleotides in the presence of different cations are shown in Figure 5. It can be seen that all the oligonucleotides with non-nucleosidic linkers generate CD signatures with a positive maximum ~264 nm and a minimum ~244 nm, which is characteristic of parallel quadruplex formation. In contrast the human telomeric sequence (GGG-TTA) has maxima at ~290 nm. The ellipticity of GGG-TTA at 290 nm is much greater in the presence of potassium than sodium and this is accompanied by a shoulder ~270 nm, while antiparallel (intramolecular) complexes usually possess a positive maximum at 295 nm and a negative minimum at 265 nm (43–46).

**Discussion**

The results presented in this paper demonstrate that oligonucleotides with non-nucleosidic linkers can adopt secondary structures, which are consistent with quadruplex formation. In each case the Tₘ values are higher in potassium than sodium containing buffers and both these cations are much more effective than lithium. However, the melting temperature, the shape of the melting profile and the kinetics of quadruplex formation are affected by the nature of the linker.
In general, the guanines adopt an anti configuration in parallel and antiparallel structures, and an alternating anti/syn arrangement in with antiparallel strands, though several different combinations of glycosidic torsion angles have been described.

The stability of the sequences that contain a single non-nucleosidic linker in each loop decreases in order GGG-Oct > GGG-H1 > GGG-Prop, in both sodium and potassium containing buffers, and these are all more stable than the human telomeric repeat sequence GGG-TTA. All three of these sequences produce stable complexes in the presence of lithium alone and there is little or no hysteresis in their melting and annealing profiles under any of the conditions examined. The shallower melting profile of GGG-Prop and its smaller dependence on potassium concentration suggest that this folded sequence contains fewer G-quartets. The linker in this sequence is the same length as that in the HIV integrase aptamer (G3T)4, which contains a single T between each of the G3 tracts (30–32). Despite the similarity between these sequences GGG-Prop is less stable, suggesting that stacking of bases in the loops plays a significant part in quadruplex stability. The loops may also affect the coordination of metal ions, since GGG-Prop is less affected by the potassium ion concentration. The loop in GGG-H1 is only slightly longer than that in the human telomere sequence, yet it forms a more stable quadruplex and displays much faster kinetics of folding and unfolding (no hysteresis). This could be because of the reduced charge (it contains only two phosphates in each linker, instead of four), but also may reflect the presence or absence of base stacking.

Although there is no hysteresis with GGG-Prop, GGG-Oct and GGG-H1, the faster kinetics is not merely a property of oligonucleotides that contain non-nucleosidic linkers as the most pronounced hysteresis is observed with GGG-H2. Clearly the formation of the G-quartets alone cannot account for this slow step, which presumably results from a metal ion-dependent rearrangement of the loops. The interaction with the metal ion does not seem to be responsible for the slow kinetics since the potassium dependency of the Tm8 for GGG-H1, GGG-TTA and GGG-H2 is very similar, and each complex appears to contain three specially bound potassium ions. It is possible that these kinetic differences are related to the different structures that have been proposed in the presence of sodium and potassium (13).

The observation that GGG-Oct has the highest stability, under all conditions, suggests that this linker is the optimal length for quadruplex formation. However, this oligonucleotide produces melting profiles with an unusual biphasic shape. This effect has been noted previously for the intramolecular quadruplex formed by oligonucleotide (G3T2)6 (39), which has the same number of bonds, but one more phosphate in each linker. The biphasic profiles suggest that these oligonucleotides fold to form several stable structures, possibly with different numbers of stacked guanines. For GGG-Oct the first part of the melting curve is very shallow, suggesting that this represents dissociation of a structure that contains fewer stacked G-quartets. This structure might contain two quartets, with G-Oct (or Oct-G) in each loop, while the more stable structure could consist of three stacked G-quartets with a single octanediol residue in each loop.

Previous studies have shown that the bases between the G-tracts can significantly affect quadruplex stability (29,39),
and demonstrates that these are not simply neutral linkers. For example, changing the loops from TTA to AAA abolishes quadruplex formation. The present study shows that their complete removal, replacing them with non-nucleosidic linkers, affects quadruplex stability. However, while GGG-Prop appears to be less stable than G3T2, and GGG-Oct has a similar stability to G3T2, and GGG-H1 is more stable than GGG-TTA, even though each pair has a similar length loop. It appears that for short loops the presence of bases enhances quadruplex stability, while longer loops are more stable with non-nucleosidic linkers. It should also be noted that the hexaethylene glycol linker might also coordinate a potassium ion (since this can adopt a similar structure to a crown ether). This work was supported by a grant from the European Union.

REFERENCES


