

G-Wires: Self-Assembly of a Telomeric Oligonucleotide, d(GGGGTTGGGG), into Large Superstructures

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ABSTRACT: The telomeric DNA oligonucleotide 5'-G₄T₂G₄-3' (Tet 1.5) spontaneously assembles into large superstructures we have termed G-wires. G-wires can be resolved by gel electrophoresis as a ladder pattern. The self-association of Tet 1.5 is noncovalent and exhibits characteristics of G4-DNA, a parallel four-stranded structure stabilized by guanine tetrads. Formation of G-wires is dependent upon the presence of Na⁺ and/or K⁺, and once formed, G-wires are resistant to denaturation. The results described here extend our understanding of the structural potential of G-rich nucleic acids and may provide insight into the possible roles of G-rich sequences and the novel structures they can form in biological systems.

Telomeres are specialized protein–DNA complexes at the ends of linear eukaryotic chromosomes. They are responsible for preserving chromosome structural integrity and stability, ensuring complete replication of chromosomal termini, and associating chromosomes with the nuclear matrix (Blackburn, 1991; Henderson & Larson, 1991; Price, 1992). In most cases, telomeric DNA consists of short repetitive sequences containing a guanine-rich strand running 5' to 3' toward the end of the chromosome and a complementary cytosine-rich strand (Greider, 1991; Zakian et al., 1990). At the very end of the chromosome, the guanine strand forms a 3' overhang of approximately two sequence repeats (Henderson & Blackburn, 1989; Klobutcher et al., 1981; Pluta et al., 1982). In many instances, telomeric repeat sequences from diverse species are very similar in composition. Examples of telomeric sequences are TTAGGG in vertebrates and trypanosomes, TTTTGGGG in the ciliates *Oxytricha* and *Euplotes*, TTGGGG in *Tetrahymena*, and TG_{1–3} in *Saccharomyces cerevisiae*.

In the presence of monovalent cations, guanosine monophosphates, guanosine derivatives (Guschlbauer & Chantot, 1976), and guanine-rich oligonucleotides (Sundquist, 1991) including telomeric sequences have the capacity to form higher order structures. These higher order structures are stabilized by G-quartets; planar structures composed of four Hoogsteen base-paired guanines in a cyclical array (Henderson et al., 1987; Sen & Gilbert, 1988; Williamson et al., 1989; Sundquist & Klug, 1989; Kang et al., 1992; Smith & Feigon, 1992). Many G-rich telomeric oligomers can form a family of related structures containing G-quartets (G-DNA). For example, a single repeat of the *Tetrahymena* telomeric G-strand sequence TGGGGT will adopt a four-stranded parallel quadruplex (Aboul-ela et al., 1992) while two repeats, (T₂G₄)₂, can adopt either a four-stranded parallel quadruplex known as G4-DNA or an anti-parallel hairpin dimer quadruplex known as G'2-DNA (Sen & Gilbert, 1990). For the two repeat molecules, the preference of one conformation over the other is most influenced by the monovalent cation present (Na⁺ or K⁺) and DNA concentration. The ability of different monovalent cations to affect G-DNA conformation has been termed the Na⁺/K⁺ switch (Sen & Gilbert, 1990).

Stabilization of G-quartets by the class Ia cations is ranked K > Rb > Na > Li = Cs. The class IIa metal cations are

also capable of stabilizing G-quartets and are ranked Sr > Ba > Ca > Mg (Venczel & Sen, 1993). The balance between the kinetics of structure formation and the stability of a structure is an important factor in determining which conformation will be favored. G'2-DNA, the anti-parallel hairpin dimer quadruplex (Sen & Gilbert, 1988), is less stable than G4-DNA but faster forming, while the tetrameric parallel-stranded quadruplex is more stable but slower to form (Lu et al., 1993). K⁺ facilitates the formation of G'2-DNA by G-rich sequences due to its superior stabilizing ability. However, in Na⁺ the same sequences will adopt the G'2-DNA conformation but will also be found in the more stable G4-DNA conformation (Sen & Gilbert, 1990). The length of the G-rich oligomer also affects which conformation it will adopt. Specifically, single repeats of telomeric G-strand sequences are too short to fold back into hairpins and, therefore, can only adopt the G4-DNA conformation.

Superstructures formed by G-rich DNAs have been observed in the presence of K⁺. An oligomer with a G-rich telomere-like sequence at its 3' end and a 5' T-tail can form G4-DNA superstructures (Sen & Gilbert, 1992; Lu et al., 1992). The superstructures are composed of 8, 12, and 16 strands (G8, G12, and G16 DNA). G4-DNA with a 3' overhang of a single G promoted formation of head to tail intermolecular structures (Sen & Gilbert, 1992). The limited number of superstructures that were obtained may have resulted from steric hindrance caused by the protruding 5' T-tails. Formation of these superstructures usually required high salt concentrations and the oligonucleotide at concentrations up to 7.5 μg/μL. It is important to note that superstructure formation was achieved in a buffer containing KCl but, in contrast to the data presented here, not in a buffer containing NaCl.

The oligonucleotide 5'-GGGGTTGGGG-3' (Tet 1.5) mimics the 3' overhang from *Tetrahymena thermophila* (Henderson & Blackburn, 1989). Here, we report the self-assembly of Tet 1.5 into long linear structures termed G-wires. Formation of these large structures is facilitated by the presence of blocks of guanines at both the 5' and 3' ends of Tet 1.5. This is the first demonstration of spontaneous self-assembly of a telomeric sequence into large polymers (i.e., greater than G16-DNA) and may provide new insight into the form and function of G-rich sequences in biological systems.

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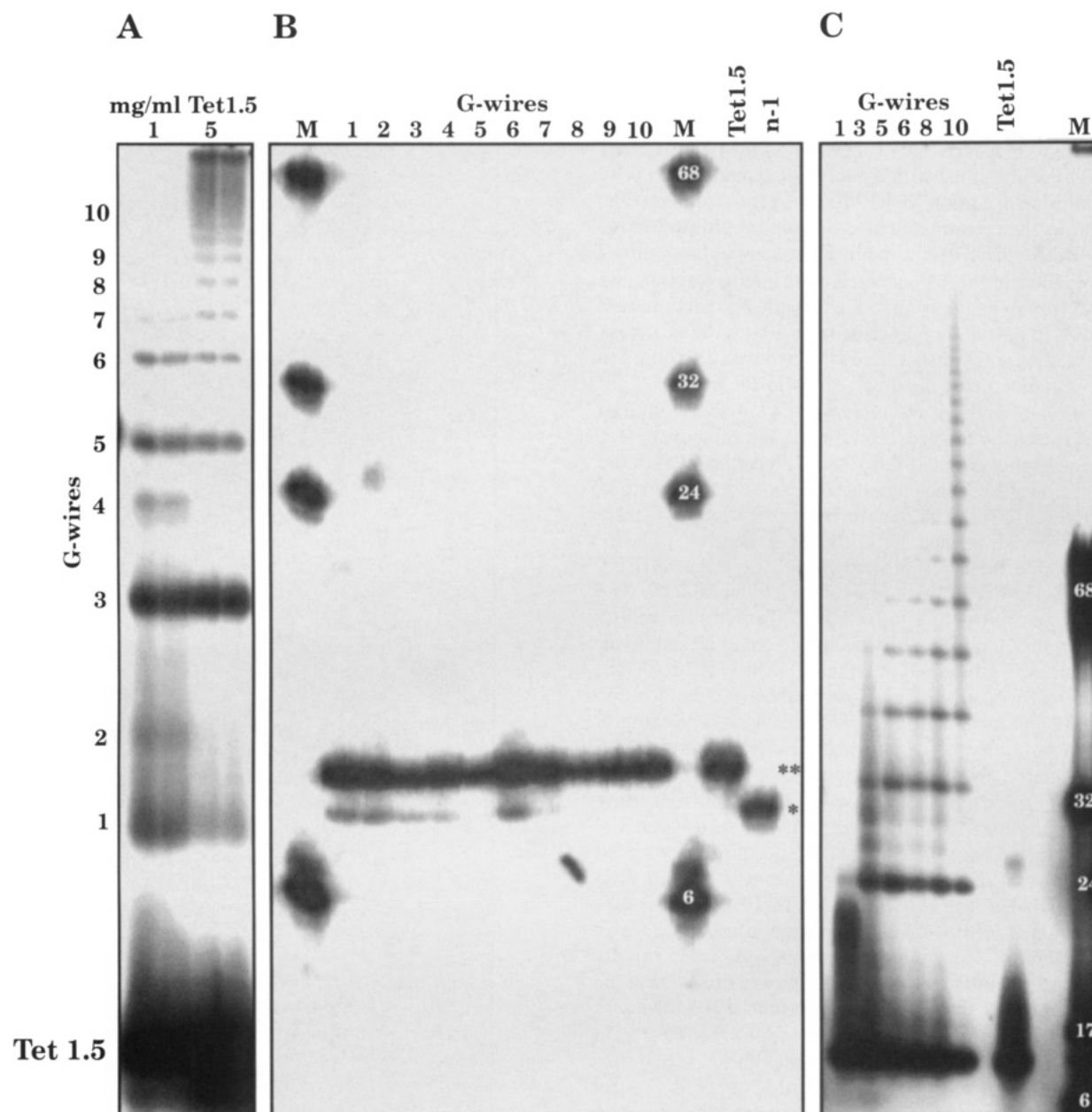


FIGURE 1: (A) Nondenaturing gel showing G-wire banding pattern after labeling Tet 1.5 at the 5' end with ^{32}P . The concentrations of oligonucleotide were 1 and 5 $\mu\text{g}/\mu\text{L}$. The individual bands are numbered 1–9 while 10 is the collection of all the lowest mobility G-wires. (B) Denaturing gel electrophoresis of the purified G-wires. Desalted G-wires were boiled for 10 min in 60% formamide loading buffer, loaded on a 20% acrylamide, 8 M urea, and 0.6 \times TBE gel, and run at 50 V/cm. The isolated G-wires bands are listed as 1–10. Tet 1.5 (10-mer) is indicated by ** and Tet 1.4 (9-mer) is indicated by * on the gel. (C) Native electrophoresis of individual isolated G-wire bands on a 10% acrylamide and 0.6 \times TBE gel run at 10 V/cm and 4 $^{\circ}\text{C}$. The G-wires examined were loaded in 50 mM NaCl, 40% sucrose, 0.6 \times TBE, 0.1% bromophenol blue, and 0.1% xylene cyanole FF. Abbreviations: M, oligonucleotide size marker, n-1, Tet 1.4; Tet 1.5, 5'- ^{32}P end labeled Tet 1.5.

MATERIALS AND METHODS

Synthesis and Purification of Oligonucleotides. Oligonucleotides were synthesized using standard phosphoramidite chemistry on an ABI 320 oligonucleotide synthesizer, boiled for 10 min in 53 mM Tris-HCl, 53 mM boric acid, 0.9 mM EDTA (0.6 \times TBE, final pH 8.3), and 40% formamide, and separated at 50 V/cm on a 20% acrylamide, 8 M urea, and 0.6 \times TBE gel. Oligonucleotide bands were visualized by UV shadowing against an intensifying screen and cut from the gel. DNA was eluted by passive diffusion from the gel fragments in 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA (TE). The oligonucleotide was desalted and concentrated by C18 (Waters) column chromatography and eluted in 50:50 methanol:water. The eluant was lyophilized and dissolved in ddH₂O.

Nondenaturing gel electrophoresis was performed at either 4 $^{\circ}\text{C}$ or room temperature (18–20 $^{\circ}\text{C}$) in 10% acrylamide and 0.6 \times TBE gels with a field strength of less than 10 V/cm. Gel bands were quantitated using a PhosphorImager (Molecular Dynamics).

5' End and Internal Labeling of Tet 1.5. Tet 1.5 was radiolabeled either at the 5' end or internally near the 3' end with ^{32}P . The 5' end of Tet 1.5 was labeled using the forward reaction of T4 polynucleotide kinase and [γ - ^{32}P]ATP at 37 $^{\circ}\text{C}$ for 30 min. The concentration of oligonucleotide in the reactions was typically 2 $\mu\text{g}/\mu\text{L}$ in a total volume of 20 μL . T4-polynucleotide kinase reaction buffer (referred to as KB throughout the text) conditions were 50 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 0.1 mM EDTA, 0.1 mM spermidine, and 0.1 mM DTT.

The internal 3' end of Tet 1.5 was radiolabeled using the T4 DNA polymerase replacement synthesis reaction [adapted from Maniatis et al. (1982)] to incorporate ^{32}P into the oligonucleotide. Tet 1.5 was annealed to an equimolar amount of complementary $(\text{C}_4\text{A}_2)_2$ (500 nM in a 20- μL reaction volume) in 50 mM Tris-HCl (pH 8.0), 50 mM MgCl_2 , and 1 mM DTT and treated with T4 DNA polymerase at 11 °C for 1 min prior to adding [$\alpha\text{-}^{32}\text{P}$]dGTP to the reaction. The reaction was then terminated after 10 min by adding 1 μL of 0.5 M EDTA (pH 8.0) and boiling the sample for 5 min.

The products of the T4 polynucleotide kinase reaction and the T4 DNA polymerase replacement synthesis reaction were gel purified in the manner described above with the exception that autoradiography rather than UV shadowing was used to locate the radiolabeled oligomer bands in the gel.

Inhibition of G-Wire Assembly by 5' Phosphorylation of Tet 1.5. Internally labeled Tet 1.5 and nonradiolabeled Tet 1.5 were phosphorylated at the 5' end with cold ATP at room temperature for 2 h. The oligonucleotides were then purified as described above. The denaturing gels included Tet 1.5 that was 5' end labeled with ^{32}P as a control. The 5' phosphorylated, internally labeled Tet 1.5 was mixed with 2.5 μg of unlabeled Tet 1.5 with or without a 5' phosphate. The samples were diluted to 10 μL of H_2O , heated in a boiling water bath for 10 min, rapidly cooled on ice and then dried. The samples were dissolved in either 50 mM NaCl KB (KB, kinase buffer) or 50 mM KCl KB to a final DNA concentration of 100 μM and incubated at 37 °C for 24 h. The samples were then examined by denaturing gel electrophoresis on a 15% acrylamide gel at room temperature.

Sample Preparation. The following description for setting up the incubations using nonradioactive Tet 1.5 and the internally labeled Tet 1.5 was standard procedure unless stated otherwise. A 1- μL sample of low concentration (10 nM) internally labeled Tet 1.5 was added to 10 μg of Tet 1.5, diluted in 10 μL of 0.1 \times TE, and heated in a boiling water bath for 10 min. The denatured sample was cooled rapidly on ice, then dried by vacuum evaporation, and dissolved in the appropriate buffer to a final concentration of 0.6 mM.

Monovalent Cation-Selective Stabilization of G-Wires. Tet 1.5 (2 $\mu\text{g}/\mu\text{L}$) was 5' end labeled and then diluted with buffer containing either 50 mM KCl KB or 50 mM NaCl KB. Control samples did not have any additional salt added to them. The samples were diluted 1:1 in a loading buffer (80% formamide, 0.6 \times TBE, 0.2% bromophenol blue, 0.2% xylene cyanole FF) containing either 50 mM NaCl or 50 mM KCl and divided into two tubes. Half of the sample was loaded directly onto a 20% acrylamide, 8 M urea, and 0.6 \times TBE gel, and the second half of the sample was boiled prior to loading on the gel.

Temperature of Incubation and Formation of G-Wires. Internally labeled Tet 1.5 was resuspended in either 50 mM NaCl KB or 50 mM KCl KB at 4, 25, 37, and 65 °C for 24 h. Samples were overlaid with mineral oil to prevent evaporation. An equal volume of loading buffer was added to the samples, which were then separated on denaturing and nondenaturing 10% acrylamide gels.

RESULTS

Generation of G-Wires. Denaturing gel electrophoresis of 5' phosphorylated Tet 1.5, which had been boiled in 40% formamide, revealed a regular pattern of bands (Figure 1A). The bands represent DNA superstructures that are resistant to moderate denaturation. The incremental, regular size increase suggested that a monomer unit was added at one or

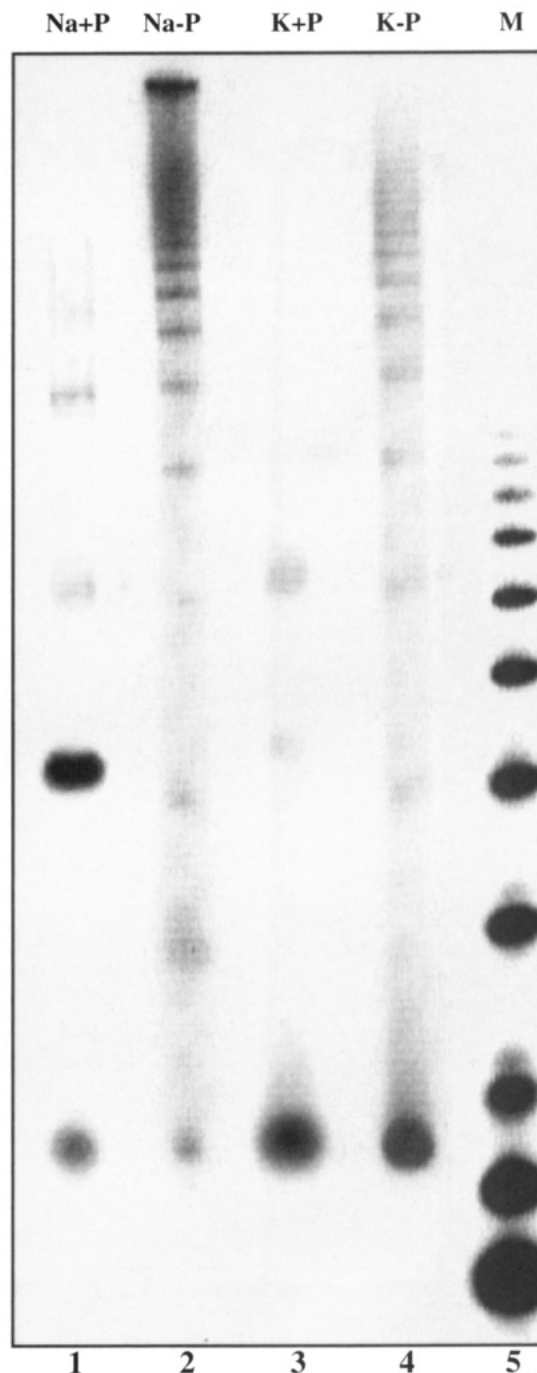


FIGURE 2: Inhibition of G-wire growth by 5' phosphorylation. Samples incubated at 37 °C for 24 h were mixed with an equal volume of 40% formamide loading buffer and run at 5 V/cm and room temperature on a 15% acrylamide, 8 M urea, and 0.6 \times TBE gel. Lanes 1 and 3 contain internally labeled 5'-Tet 1.5, and lanes 2 and 4 are internally radiolabeled Tet 1.5 lacking a 5' phosphate. The experiments were analyzed on different gels but carried out under identical conditions. Abbreviations: Na, 50 mM NaCl KB; K, 50 mM KCl KB; +P, 5' phosphorylated Tet 1.5; -P, non-5' phosphorylated Tet 1.5; M, ligated *Kpn*I linkers of 8-bp increments.

both ends of the structures. These higher order structures are referred to as G-wires.

To confirm that the G-wires were composed of Tet 1.5 monomer oligonucleotide, individual G-wire bands were cut from the gel, eluted, and desalted as described in Materials and Methods. The desalted G-wire bands were denatured in 60% formamide loading buffer and separated on a denaturing gel. All of the G-wire bands were composed of the Tet 1.5 oligonucleotide (Figure 1B). A small percentage of the 9mer

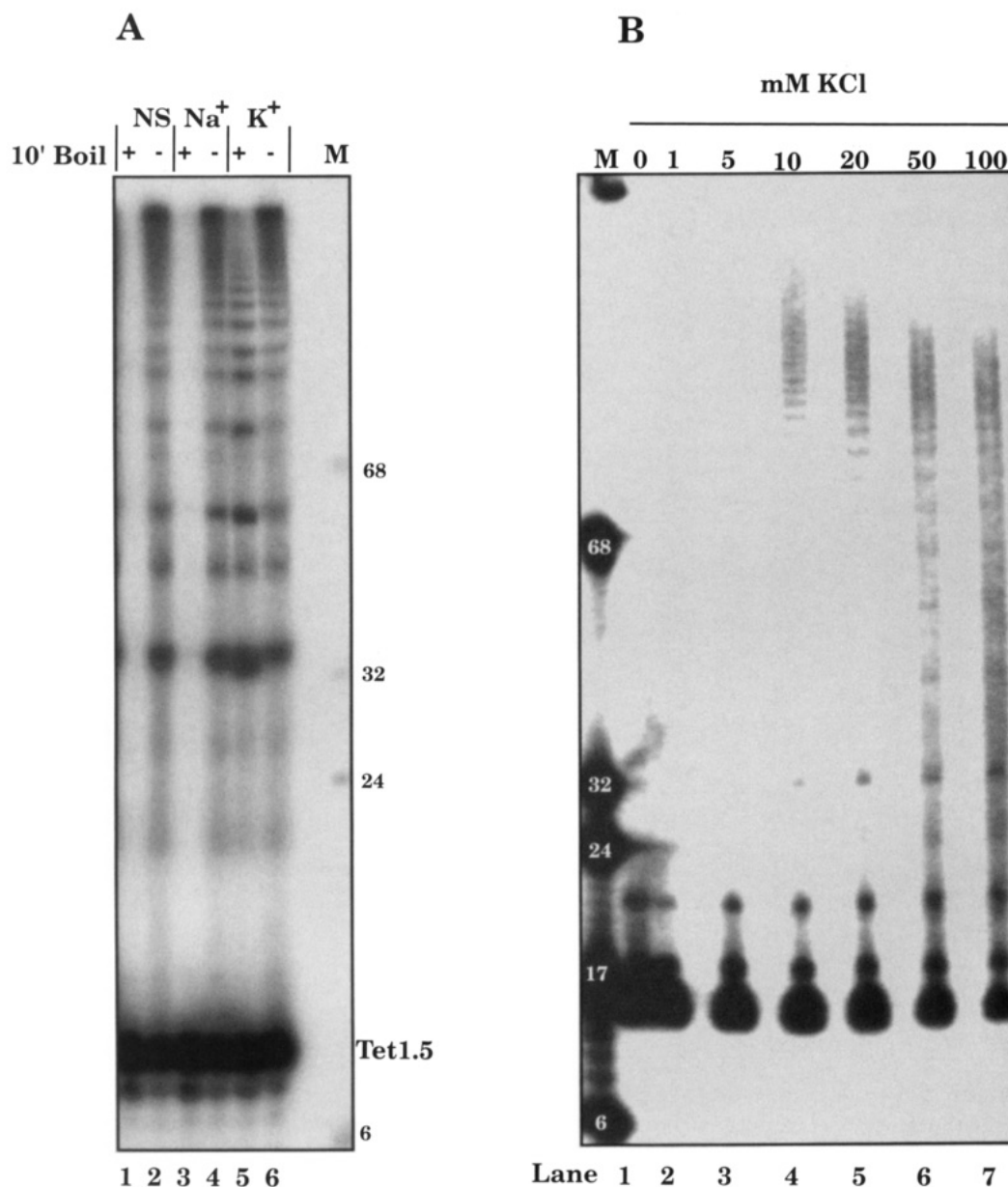


FIGURE 3: Potassium stabilization of G-wires. (A) G-wires grown in the presence of 50 mM NaCl KB were incubated with 50 mM KCl KB 10 min prior to boiling in 40% formamide loading buffer and separation by gel electrophoresis. Denaturing gel electrophoresis shows the selective stabilization and maintenance of the Na⁺ G-wire banding pattern by K⁺. (B) Denaturing electrophoresis of G-wires grown in 50 mM NaCl buffer and then treated for 1 h with an increasing concentration of KCl prior to boiling and separation on a denaturing gel. Samples were run on a 20% acrylamide, 8 M urea, and 0.6× TBE gel at 50 V/cm.

premature termination product d(GGGGTTGGG) (Tet 1.4) was associated with the purified G-wire bands. The proportion of Tet 1.5:Tet 1.4 increased with the increase in G-wire size, suggesting that Tet 1.4 could be involved in the termination of G-wire growth.

To test the stability of the G-wires, purified G-wire bands were isolated and eluted from acrylamide gels. Upon analysis of single isolated bands by electrophoresis, the banding pattern that was originally observed was to a large extent regenerated. The maximum size of G-wires in the regenerated banding pattern was related to the original size of the purified G-wire but was slightly greater than that band size (Figure 1C). In addition, the amount of monomeric Tet 1.5 decreased as the size of the G-wire increased.

5' Phosphate Inhibition of G-Wire Formation. We were unable to obtain G-wire formation from Tet 1.5 that was homogeneously 5' phosphorylated (Tet 1.5 monomer band in Figure 1A), suggesting that the presence of a 5' phosphate on

Tet 1.5 hindered G-wire formation. To test this possibility, internally radiolabeled Tet 1.5 was phosphorylated on the 5' end using nonradiolabeled ATP and incubated under conditions conducive to G-wire growth. As seen in Figure 2, the presence of a 5' phosphate group inhibited G-wire formation in both Na⁺ and K⁺ (Figure 2, lanes 1 and 3). Some structures did form in Na⁺, but these were distinct from the regular banding pattern G-wires display. Therefore, the presence of a 5' phosphate group has a significant detrimental effect on the formation of G-wires.

Monovalent Cation-Dependent Assembly of G-Wires. To test the hypothesis that G-wires are G-DNA structures, we examined their growth and stability in Na⁺ and K⁺. A feature of G-DNAs in general is stabilization by the monovalent cations with K⁺ conferring greater stability than Na⁺. G-wires grown in the presence of 50 mM NaCl and then incubated for 10 min in either 50 mM NaCl, 50 mM KCl, or no additional salt showed a dramatic difference in resistance to denaturation

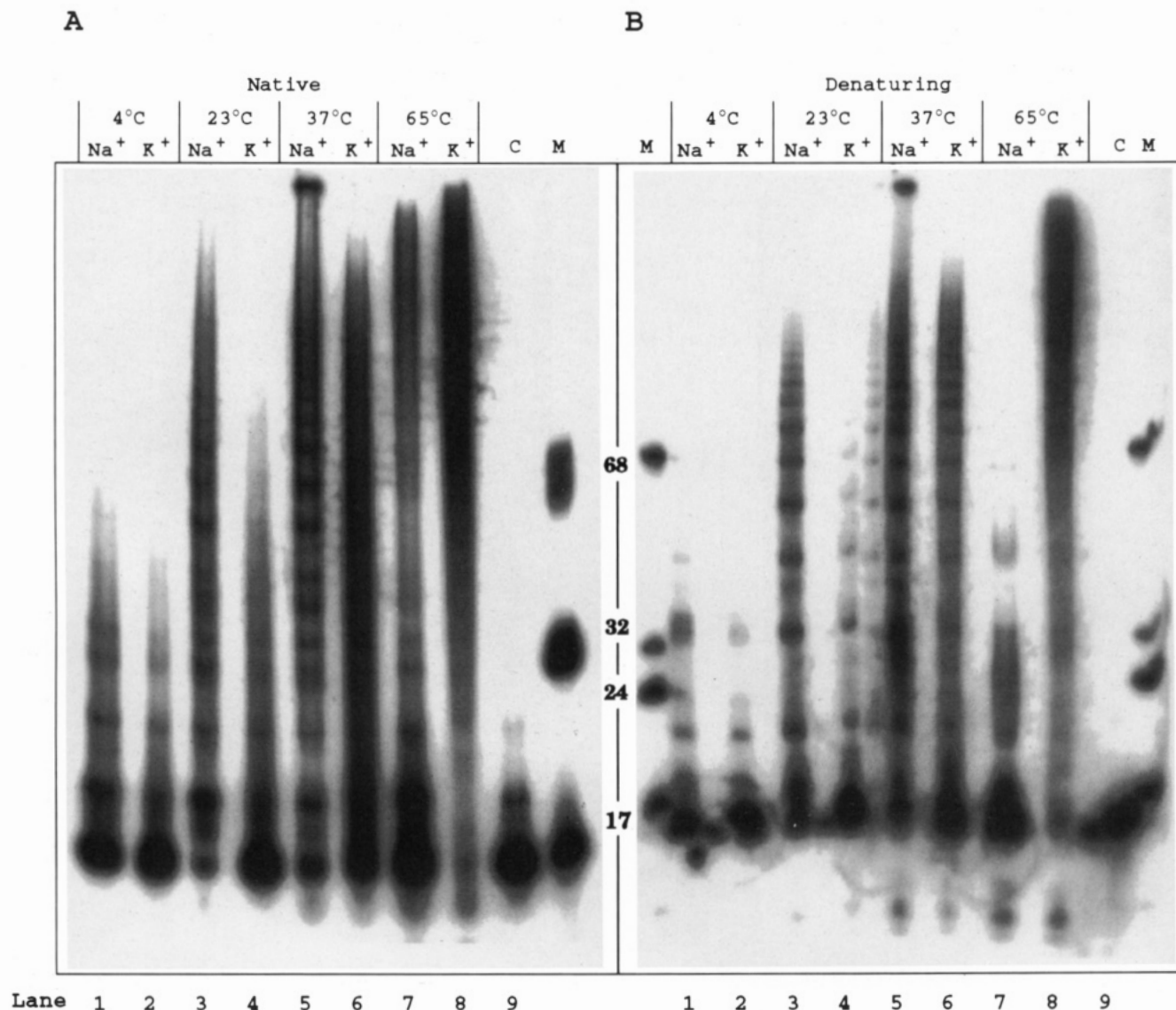


FIGURE 4: Temperature dependence of G-wire formation. (A) Native 10% acrylamide gel run in 0.6× TBE. Samples were either incubated in 50 mM NaCl buffer or 50 mM KCl buffer at the indicated temperatures. Prior to loading, the samples were divided into equal portions and mixed 1:1 with the appropriate loading buffer. (B) Denaturing gel of the temperature incubation samples. Abbreviations: C, Tet 1.5 in the absence of additional cation; M, oligonucleotide size markers.

when boiled in 40% formamide and examined by denaturing gel electrophoresis (Figure 3A). The boiled sample treated with K⁺ was highly resistant to denaturation (Figure 3A, lane 5); however, the G-wire samples treated with Na⁺ or no additional salt were totally denatured (Figure 3A, lanes 3 and 1, respectively). Interestingly, longer treatment with 50 mM KCl gave a different result. G-wires were grown in 50 mM NaCl then treated for 1 h in KCl concentrations that ranged from 0 to 100 mM. The treated samples were boiled in 40% formamide and examined by denaturing gel electrophoresis (Figure 3). Treatments with as little as 5 mM KCl were capable of providing some resistance to denaturation (Figure 3B, lane 3). Smearing of the G-wire banding pattern occurred in the 50 and 100 mM KCl treatments (Figure 3B, lanes 6 and 7).

Temperature-Dependent Formation of G-Wires. Tet 1.5 was incubated in the presence of either 50 mM NaCl KB or 50 mM KCl KB at 4, 23, 37, and 65 °C for 24 h. The growth of G-wires was detected by native (Figure 4A) and denaturing (Figure 4B) electrophoresis. The samples incubated in NaCl showed a marked improvement in G-wire formation with increasing temperature, peaking at 37 °C. At 65 °C, there

was very little G-wire formation in Na⁺. Comparing the native and the denaturing gels for the Na⁺ grown G-wires, it was observed that they had a more complex banding pattern under nondenaturing conditions. Apparently denaturation removed the less stable conformations contributing to the complexity of the banding pattern observed under nondenaturing conditions. Thus, comparison of native and denaturing gels reveals the most stable structures, i.e., perfectly aligned G-wires.

The samples incubated in K⁺ showed little growth of G-wires at 4 or 23 °C. Under nondenaturing conditions, incubation at 37 and 65 °C in K⁺ created a large smear of irregular higher order structures. The elimination of less stable conformations under conditions of denaturing electrophoresis revealed a G-wire banding pattern like that observed in the presence of Na⁺. In addition, an apparent compression of the K⁺ banding pattern is observed at 65 °C under denaturing conditions.

These results show that it is possible to manipulate the incubation conditions to generate G-wires in either Na⁺ or K⁺ and that the temperature at which G-wires form depends on the type of monovalent cation that is present.

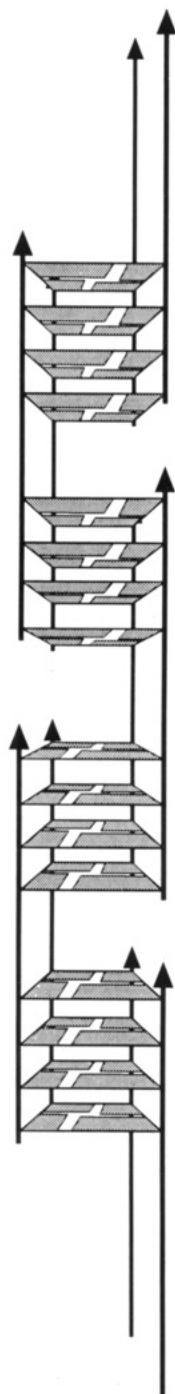


FIGURE 5: Proposed G-wire model. Tet 1.5 adopts an out of register (slipped) tetrameric association forming G4-DNA domains stabilized by monovalent cations. The overlapping slipped G4-DNA structures are responsible for the formation of the G-wire. This model is speculative but consistent with the data presented herein.

DISCUSSION

General Summary. The telomeric oligonucleotide d(GGG-GTTGGGG) (Tet 1.5) is capable of spontaneous self-assembly into superstructures that are most likely stabilized by G-quartets. These structures, termed G-wires, can be resolved as a regular pattern of bands by gel electrophoresis. Growth of G-wires with Tet 1.5 is much more extensive and occurs at lower cation concentrations than that of previously reported G-DNA superstructures (Sen & Gilbert, 1992; Lu et al., 1992).

Hypothetical G-Wire Structure. On the basis of observations presented here, we propose a model that represents the most probable G-wire conformation (Figure 5). In this model,

the G-wire consists of G4-DNA domains punctuated by T nodes. The initial starting structure consists of a G4 domain containing four quartets formed by the association of the 5' half of a duplex Tet 1.5 with the 3' half of another Tet 1.5 duplex, forming a slipped tetraplex structure with G-duplex "sticky ends". The strands run parallel to each other and can accept additional duplex Tet 1.5 at either end. The slipped tetraplex association of Tet 1.5 is consistent with the regular ladder pattern seen by gel electrophoresis. Sen and Gilbert (1992) proposed a slipped architecture for the superstructures they observed that is conceptually similar to that proposed here for Tet 1.5. The increased stacking interactions would enhance the stability of the G-wire.

G-wires Have G-DNA Characteristics. In the presence of Na^+ and K^+ , Tet 1.5 assumed different conformations. At low temperatures, K^+ stabilized conformations that did not lead to G-wire formation. These structures were most likely hairpin dimers (G'2-DNA), which is consistent with previous reports (Sundquist & Klug, 1989; Sen & Gilbert, 1990). Although Na^+ may facilitate the formation of the same conformations as K^+ , it may not stabilize them as well as K^+ . Therefore, many thermodynamically less favored structures (e.g., G'2-DNA) are short lived in Na^+ compared to K^+ while the more stable four-stranded structure, G-4 DNA (Lu et al., 1993), predominates, leading to the formation of G-wires.

Assembly of G-wires occurred most efficiently in Na^+ , yet a greater degree of stability was acquired by the addition of K^+ . A similar phenomenon was reported for the association of chromosomal DNA containing telomeric repeats (Oka & Thomas, 1987) and for a yeast telomeric G-strand oligonucleotide (Venczel & Sen, 1993). Growing G-wires in Na^+ followed by a brief incubation in K^+ vividly demonstrated the strong stabilizing effect of K^+ on G-wires. What is most striking about this is the resistance to thermal denaturation and imperviousness to denaturing agents (8 M urea and 40% formamide). The stabilizing effect was only observed for brief (i.e., less than 30 min) treatments with K^+ . Exposing G-wires to K^+ for longer periods of time resulted in their rearrangement. Thus, G-wires are not static structures. They undergo assembly and disassembly in a relatively short time scale in comparison to some telomeric G4 structures, which can have half-lives on the order of many hours (Raghuraman & Cech, 1990). This is clearly illustrated by the regeneration of the G-wires from a single isolated band (Figure 1C). A variety of transient structures arise in solution as part of the dynamic equilibrium of G-wire assembly. Stabilization of the resulting structures by the addition of potassium would account for the smearing of the G-wires on gels.

Other Factors Influencing G-Wire Formation. The major factors that influence the formation of G-wires are the species of monovalent cation, 5' phosphorylation, DNA concentration, and the temperature at which the formation occurs. The ionic species effect was discussed in the preceding section. The 5' phosphate effect, in which the phosphorylation of Tet 1.5 inhibited the formation of G-wires, is an interesting phenomenon that is incompletely understood but likely related to steric hindrance and/or electrostatic interference between adjacent G4 domains when 5' phosphates are present.

The effect of temperature is related to the requirement for reducing the stability of structures that impede G-wire growth and allowing stabilization of the G-wire precursors. Incubation in K^+ at 65 °C and above resulted in the formation of stable high molecular weight species, the structural details of which are unknown but are likely to be irregular G-wires. These structures appear as a smear on a non-denaturing gel. Regular

G-wires (containing only blocks of 4 G-tetrads) maximize the number of possible quartets that stabilize the entire superstructure. Denaturing gel electrophoresis revealed that regular G-wires, the most stable structural species, were present within the smear of irregular structures. A similar observation was reported in which thermal denaturation of a telomeric sequence in a K^+ buffer induced structure formation as the temperature approached 95 °C (Lu et al., 1993). This is also consistent with the phenomenon of high-temperature guanosine monophosphate gel formation in the presence of K^+ and poly-G resistance to thermal denaturation (Pinnavaia et al., 1975; Zimmerman, 1976).

Biological Relevance of G-Wires. Are structures related to G-wires biologically relevant? G4 binding proteins exist in eukaryotic cells (Chung et al., 1992; Liu et al., 1993; Pearson et al., 1993; Walsh & Gualberto, 1992; Weisman-Shomer & Fry, 1993; Schierer & Henderson, 1993; Fang & Cech, 1993). It has been shown explicitly in two cases (Schieerer & Henderson, 1993; Fang & Cech, 1993) and implied in others that G4 binding proteins bind best when there is a single-stranded domain adjacent to the G4 structure. This is the case with G-wires, which are bound by a G4-DNA binding protein from *T. thermophila* (Schieerer & Henderson, 1993). This suggests that G-wires at the very least mimic some DNA conformation that may exist *in vivo*. A number of phenomenon, such as recombination, meiotic chromosome pairing, gene rearrangement, and chromosome polytenization, require self-recognition similar to that observed with G-wires and all other G4 DNA structures. Thus, the further elucidation of the structural variability of G4 DNA will shed light on its role in these and perhaps other cellular phenomena. Finally, the conformational dynamics and special features of G-wires provide new and provocative twists in the study of the biology and chemistry of self-recognizing nucleic acid structures.

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