Possibility of an Antiparallel (Tetramer) Quadruplex Exhibited by the Double Repeat of the Human Telomere†

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ABSTRACT: Under physiological concentrations of Na^+ and K^+, human telomeric DNA can self-associate into G-quadruplexes. On the basis of circular dichroism, gel electrophoresis, gel filtration, and UV-melting experiments, we report here that the double repeat of human telomere (d-TTAGGGTTAGGG; HUM2) forms parallel as well as antiparallel quadruplexes in the presence of K^+, whereas Na^+ facilitates only the antiparallel form. Here, the gel techniques and CD studies have proved to be complementary in detecting the molecularity and pattern of strand orientation. By correlating the gel and CD experiments, the antiparallel G-quadruplex was identified as a tetrameric species, whereas the parallel G-quadruplex was found to be dimeric. Both structural species were separated through gel filtration, which when run on native polyacrylamide gel electrphoresis (PAGE), confirmed their molecularity. UV-melting profiles also confirm the presence of two biphasic and one monophasic structural species in the presence of K^+ and Na^+, respectively. Though our observation is consistent with the recent NMR report (Phan, A. T., and Patel, D. J. (2003) *J. Am. Chem. Soc. 125*, 15021−15027), it seems to differ in terms of the molecularity of the antiparallel quadruplex. A model is proposed for an antiparallel tetrameric quadruplex, showing the possibility of Watson−Crick hydrogen bonds between intervening bases on antiparallel strands. This article expands the known structural motifs of DNA quadruplexes. To the best of our knowledge, four-stranded antiparallel quadruplexes have not been characterized to date. On the basis of the model, we hypothesize a possible mechanism for telomere−telomere association involving their G-overhangs, during certain stages of the cell cycle. The knowledge of peculiar geometries of the G-quadruplexes may also have implications for its specific recognition by ligands.

Telomeres are specialized structures comprising DNA and protein, capping the ends of eukaryotic chromosomes. The possible functions of telomeres include maintaining the structural integrity of chromosomes, ensuring complete replication of their extreme ends, and helping to establish the three-dimensional architecture of the nucleus and/or chromosome pairing (*1*−*3*). The G-rich strand of the telomere has a single-stranded extension toward the 3′-end and can be written in a general form as $G_n(A/T)_m$ when $n > 1$ and $m = 1-4$. The resulting single-stranded overhang, or telomere tail, is capable of forming complex intrastranded associations (*4*). In addition to telomere ends of eukaryotic chromosomes, runs of G's may also be found in other locations including the *c-myc* promoter (*5*), the triplet repeat region that can cause a variety of neurological disorders (*6*), the recombination and mutation hot spots (*7*), and the switch region of immunoglobins (*8*).

Telomerase, a ribonucleoprotein complex which ensures replication of the telomeres, may be proposed as attractive targets for the discovery of new anticancer agents (*9*). A number of small molecules have been discovered to inhibit the function of telomerase by stabilizing G-quadruplex structures (*10*−*11*). Davis (*12*) has elegantly reviewed the significance of G-quartet assemblies in areas ranging from structural biology and medicinal chemistry to supramolecular chemistry and nanotechnology.

It is now well established that the G-rich sequences can self-associate *in vitro* to form different types of DNA quadruplexes, all containing guanine base tetrads or quartets (G-tetrad); planar structures composed of four Hoogsteen base paired guanines in a cyclic array, thanks to the multiple hydrogen bonding donor and acceptor sites of the nucleobase guanine, which makes it "sticky" (*12*). Many laboratories have reported that DNA oligonucleotides having repetitive tracts of guanine bases can form G-quadruplex structures that display an amazing polymorphism under cellular environmental conditions, such as pH, cations, and temperature. Monovalent cations, notably K^+ and Na^+, greatly stabilize G-quadruplex structures presumably by coordinating with eight carbonyl oxygens sandwiched between two coplanar quartets. The structure of synthetic oligonucleotides corresponding to telomeric G-rich strands has been discussed extensively in recent, excellent reviews (*13*−*16*). Several structures have been identified, and these include four-stranded intermolecular quadruplexes, hairpin dimers, and monomolecular intramolecularly folded quadruplexes (*14*, *17*, *18*). There are a number of ways in which four strands of DNA may interact and pair into a stable structure. They

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can be all parallel, three parallel and one antiparallel, or with two pairs of adjacent parallel strands and with alternating antiparallel strands (Figure 6a) (*15*, *18*). Though the quadruplex structures seem to be overwhelmingly polymorphic, the G-tetrad is conserved as the basic structural motif. It had been assumed that the quadruplex with all strands parallel was particular to intermolecular quadruplexes and that all quadruplexes involving one or two telomeric strands would adopt one of the above said three antiparallel arrangements (Figure 6aii, iii, and iv). Antiparallel tetramolecular quadruplexes have not been described (*17*, *18*). Single tracts of guanine associate to form mostly parallel four-stranded structures. Double repeats of guanine separated by two or more of the other bases have a tendency to fold into dimeric structures. Unimolecular antiparallel quadruplexes can only be formed by oligomers that contain at least four separate stretches of two to four contiguous guanines (*14*, *16*, *19*). The ends of all human chromosomes consist of a repeated non-coding d-$(TTAGGG)_n$ DNA sequence (*20*). Existing studies have focused primarily on telomeric sequences in which the intervening bases have been T or T, A (*21*). Tiny telomere DNA constructs have been studied (*22*), which provide a model to study the duplex−quadruplex junction region at the end of chromosomal region. The single-, double-, and four-repeat sequences of the human telomere have been extensively studied by X-ray, NMR, and other spectroscopic and biochemical methods (*21*, *23*−*26*). Recently, fluorescence resonance energy transfer (FRET) has also been applied to study DNA quadruplex formation (*27*, *28*).

The NMR structure of G-quadruplexes formed by single and four repeats of the human telomere sequence was reported several years ago (*24*, *25*). The single copy TTAGGG formed a parallel four-stranded structure in K^+ with all of the guanine in anti-conformation, whereas the four repeats (almost) of same sequence in a 22-mer d-$[AGGG(TTAGGG)_3]$ adopted the intramolecular quadruplex structure in Na^+. The same four-repeat sequence in K^+ has recently been studied by X-ray crystallography (*23*). Interestingly, the oligonucleotide folded intramolecularly into a parallel propeller-type conformation with all four G-strands parallel (G-stretches oriented in same direction). The report also described the structure of double repeat d-(TAGGGTTAGGGT) as a dimeric quadruplex having the same propeller and loop architecture.

More recently, distinct topologies have been described for the two-repeat human telomere d-(TAGGGTTAGGGT) sequence in solution (*26*). This NMR study demonstrated that the said sequence can form parallel and antiparallel G-quadruplex structures in K^+-containing solutions; importantly, both the structures were found to be dimeric. Detection of a second quadruplex species in solution through NMR in contrast to X-ray studies (*23*) may be reflected in different experimental conditions used for quadruplex annealing.

As a part of our ongoing research interest in the structural studies of multistranded DNA and its interactions with ligands, we observed some interesting structural properties of the double repeat sequence of the human telomere (d-TTAGGGTTAGGG), designated as HUM2 from here on. Using gel electrophoresis, gel filtration, circular dichroism, and UV-melting experiments, we showed here that the double repeat sequence of the human telomere (HUM2) adopts parallel as well as antiparallel conformations in the presence of K^+, whereas in the presence of Na^+, the G-quadruplex species are restricted to antiparallel forms. Though our observation of coexisting parallel and antiparallel quadruplex species is consistent with the recent NMR report (*26*), it seems to differ in terms of the molecularity of the antiparallel quadruplex species, addressed here as the tetramer. Though the antiparallel G-quadruplexes composed of four strands can be imagined, to the best of our knowledge, not one has been characterized till date. A model is proposed for the antiparallel tetramer G-quadruplex stabilized by Watson−Crick hydrogen bonding between intervening bases on the antiparallel strands. A possible biological relevance of the proposed model is discussed.

MATERIALS AND METHODS

The oligonucleotides, synthesized in 1 μmol scale by Bio Basic Inc., Canada, were received in lyophilized powder form. The oligomers were supplied in purified form via 16% polyacrylamide gel electrphoresis (PAGE) in 7 M urea, supplemented with an electrophoretogram exhibiting a single band and stated purity of 99%. They were stored at −20 °C and were used without further purification. The concentration of the oligonucleotides was determined spectrophotometrically by using the extinction coefficient (ϵ) calculated by the nearest neighbor method (*29*) and by measuring the absorbance at 260 nm at elevated temperatures (90 °C), following the method described earlier (*30*). The ϵ values used for the DNA oligonucleotides d-TTAGGGTTAGGG (HUM2) and d-TTTGGGTTTGGG (HUM2C) were 147000 and 110600 M^{-1} C m^{-1}, respectively, whereas for d-CTTGAGCTCAAG (PAL) and for d-TTGGGGTTGGGG (TETRA), the values were 115940 M^{-1} C m^{-1} and 114600 M^{-1} C m^{-1}, respectively. PAL and TETRA were used as molecular size markers in gel assays. Random 35-base and 60-base single-stranded oligomers d-GACTGACTTAAGCGCATAGCTAGCTCGACTGA (M35; ϵ = 324600 M^{-1} C m^{-1}) and d-GACTGACTTAAGCGCATAGCTAGCTCGACTGAGACTGACTTAAGCGCACTAGCT (M60; ϵ = 581900 M^{-1} C m^{-1}) were also used as control size markers. The stock solutions of the oligomers were prepared by directly dissolving the lyophilized powder in MilliQ water. Reagent grade inorganic salts were purchased from Sigma and were used without further purification. The buffer solution consisted of 20 mM sodium cacodylate (pH 7.4) and 0.1 mM EDTA and were adjusted to desired ionic strength with NaCl, KCl, or $MgCl_2$.

Non-Denaturating Gel Electrophoresis. For performing gel assays, oligonucleotide samples were prepared in 20 mM sodium cacodylate buffer (pH 7.4) at desired concentrations. The final volume of the sample in the buffer was 20 μL. Importantly, prior to performing gel assays in non-denaturating conditions, the purity of the commercially made oligomers was checked by running them on 20% PAGE using 7 M urea. They all migrated as single bands; however, the G-rich oligomers, as expected, moved slower than the sequence with identical size but random base composition. For non-denaturating gel assays, the samples (20 μL) of total volume were heat treated at 95 °C for 5 min and slowly cooled to room temperature over about 10 h. The oligonucleotide (at 10 μM strand concentrations) samples were incubated at 4 °C for 3 h before loading onto 10%

polyacrylamide gel pre-equilibrated at 4 °C for 2 h. The gel contained 20 mM sodium cacodylate with 100 mM NaCl, 100 mM KCl, and 10 mM $MgCl_2$, and 0.1 mM EDTA, and the running buffer consisted of 1× TBE with 100 mM NaCl, 100 mM KCl, or 10 mM $MgCl_2$. For simplicity, the salts are designated as Na^+, K^+, or Mg^{2+} cations at appropriate places in the text. Tracking dye consisted of Orange-G or bromophenol blue. The gels were run at a constant voltage of 40 V in a cold room (4 °C). After electrophoresis, the gels were stained with ethidium bromide or Stains-all (Sigma) solution and finally visualized under UV-light/white light and photographed by Alphalmager 2200 (Alpha Infotech Corporation). Performing gel studies at room temperature (~25 °C) did not change the mobility pattern of oligomers compared to gels run in cold room. Also, identical results were obtained with staining using ethidium bromide or Stains-all.

UV-Thermal Denaturation. The UV−thermal denaturation experiments were performed on a Varian make CARY-100 spectrophotometer equipped with a Peltier thermo programmer interfaced with a Pentium III computer for data collection and analysis. The stoppered quartz cuvettes of 10 mm optical path length and 1 mL volume were used for the experiments. The cell holder was thermostated with a circulating liquid (80% water and 20% ethylene glycol). The oligonucleotide samples were prepared by taking their appropriate range of strands concentrations and heating the samples up to 95 °C for 5 min followed by slow cooling. The temperature dependence on the absorption value of the DNA (melting curves) was monitored at two wavelengths: 265 and 295 nm. The temperature of the cell holder was increased from 17 to 95 °C at a rate of 0.5 °C/min. A Teflon-coated temperature probe, immersed directly in a control cuvette, measured the sample temperature. Melting profiles were found to be irreversible, that is, the heating and cooling curves were not superimposable. This is in accord with the data obtained for rather similar systems (*31*, *32*). The thermal melting temperature (T_M) was determined from the peak of the computer generated first derivative of the thermal denaturation profile. The accuracy of the reported T_M values is ±1°C.

Circular Dichroism Spectroscopy. To obtain information on the secondary structure of the HUM2 oligonucleotide, circular dichroism (CD) spectroscopy was used. CD Spectra were recorded on a JASCO-715 spectropolarimeter interfaced with an IBM PC compatible computer calibrated with D-Camphor sulfonic acid. Five scans of the spectrum were collected over a wavelength range of 220−320 nm at a scanning rate of 100 nm/min. The average of multiple scans was used for analysis. The scan of the buffer alone recorded at room temperature was subtracted from the average scans for each DNA strand. Samples were scanned in a 1 cm path length quartz cuvette of 1 mL volume capacity. Data were collected in units of millidegrees versus wavelength and were normalized to total DNA strand concentration.

Gel Filtration Chromatography. A 1.5 × 20-cm glass column (Bio-Rad) was filled up to 16 cm with Bio-Gel P-10 (medium) gel filtration matrix (Bio-Rad) and equilibrated in a cold room (4 °C) with several column volumes of gel filtration buffer (20 mM sodium cacodylate buffer (pH 7.4), 100 mM KCl, and 0.1 mM EDTA) at a flow rate of 10−12 mL/h using a peristaltic pump. The concentrated DNA sample (200 μM) was loaded onto the equilibrated size-exclusion column, and chromatography was performed at room temperature, with the same flow rate, collecting 50−60 μL fractions. The fractions were monitored for DNA by absorbance at 260 nm. The same collected DNA fractions were finally analyzed on 10% non-denaturating PAGE.

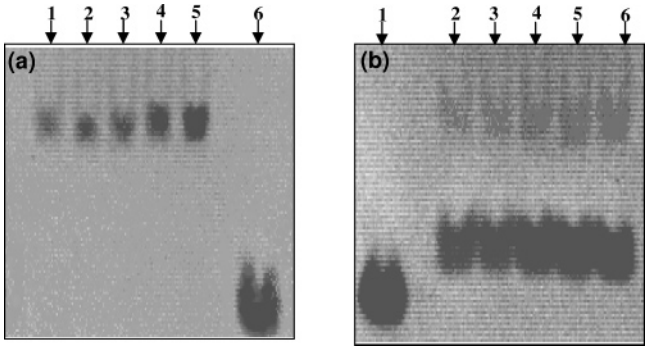

FIGURE 1: (a) 10% native PAGE mobility pattern of the oligonucleotide sequences. Lanes 1−5: HUM2 d-(TTAGGGTTAGGG) in increasing concentrations of Na^+ (50, 100, 200, 300, and 400 mM). Lane 6: PAL d-(CTTGAGCTCAAG) used as the 12-mer duplex marker. (b) 10% native PAGE mobility pattern of the oligonucleotide sequences. Lane 1: PAL d-(CTTGAGCTCAAG) used as the 12-mer duplex marker. Lanes 2−6: HUM2 d-(TTAGGGTTAGGG) in increasing concentrations of K^+ (50, 100, 200, 300 , and 400 mM).

RESULTS AND DISCUSSION

The present study reports on biophysical studies of a 12-mer oligonucleotide (HUM2, d-TTAGGGTTAGGG) consisting of two repeats of human telomere DNA. The G-quartet structure was proposed as a general model for the structure of telomeric DNA sequences (*33*, *34*). G-rich oligonucleotides can be very polymorphic with structures adopted being dependent on several factors, including base sequence, strand concentration, and the cation present. The double repeat sequence of the human telomere was studied for the quadruplex model because of its well characterized and documented structure (*23*, *26*). In our attempt to analyze the structure of the double repeat sequence (HUM2) in Na^+ and K^+ solutions at physiological pH, a novel quadruplex structure with distinct topology was identified, which extends the degree of polymorphism shown by human telomeric DNA.

Differential Electrophoretic Pattern in Na^+, K^+, and Mg^{++}. The gel electrophoresis technique is sensitive to structural conformations and has been extensively used to monitor the conversion from unfolded strands to intramolecularly folded quadruplexes, bimolecular hairpin structures (dimers), or four-stranded complexes (tetramers), and to differentiate these structures from each other (*19*). A 10% polyacrylamide native gel electrophoretic pattern of HUM2 incubated in 20 mM sodium cacodylate buffer (pH 7.4) containing increasing concentrations of NaCl ranging from 50 to 400 mM is shown in Figure 1a. The 12-mer PAL (palindromic) sequence used as a 12-base pair control (lane 6) showed a single band. HUM2 at varied concentrations of NaCl (lanes 1−5) also showed a single band, reflecting the presence of single structural species. The observed increase in band intensity with ascending concentration of Na^+ reflects

that the association is favored by an increase in monocation concentration. The exact status of HUM2 in terms of molecularity, when compared with that of the control oligomer band, was assumed to be tetrameric on the basis of its extent of retardation. Nevertheless, the possibility of the same as a dimeric form cannot be ruled out at this stage. In light of well-established previous reports, this observation seems to be intriguing. The 1.5 repeat of human telomere d-(GGGTTAGGG), in the presence of 70 mM Na^+, was shown to form an antiparallel hairpin dimer quadruplex as inferred from non-denaturating gel and CD studies, and the possibility of the two hairpin dimers stacked end to end via G-tetrad planes was also realized (*21*). Formation of the superstructure (dimer of a dimer) could be ruled out in HUM2, as only one end of the oligomer has free guanine. We reasoned that the formation of the dimer of a dimer (superstructure) is only possible with the reported truncated human telomere repeat leaving both the 5′- and 3′-ends of the oligomers with free guanines (*21*). So far, our opinion about the molecular architecture formed by HUM2 involves a structure comprising four strands. Because the two and four repeats of human telomeric sequences, in the presence of sodium, are reported to form antiparallel quadruplexes (*21*, *25*, *35*, *36*), there seems to be a fair possibility that the HUM2 tetramer is an antiparallel quadruplex structure with possible arrangements of alternating antiparallel or adjacent parallel strands (*15*, *18*).

Similarly, to characterize the structure formed by HUM2 in the presence of K^+, the samples prepared in varied concentrations of K^+ (50 mM to 400 mM) were run on a 10% non-denaturating polyacrylamide gel. The results are shown in Figure 1b. The oligomer HUM2 exhibited two distinct bands at all concentrations of K^+ (lanes 2−6). The two bands clearly appeared in the gel and indicate the presence of two structural species facilitated by K^+. This observation is in agreement with two recent reports, where the double repeat of the human telomeric sequence has been shown to coexist as parallel and antiparallel G-quadruplex structures in solutions containing K^+ (*26*, *37*). The electrophoretic mobility of the fast-moving lower band is comparable to the mobility of the 12-mer duplex of the palindromic control sequence (Figure 1b, lane 1). This indicates the possibility of the fast-moving structural species of HUM2 being present in dimeric form. It is important to mention here that though HUM2 and PAL are identical in sequence length (12 nucleotides), their dimers migrate with differential electrophoretic mobility, i.e., HUM2, being a G-rich sequence, moves slower than PAL. The presence of HUM2 existing as a dimer is in agreement with X-ray and NMR reports (*23*, *26*). The electrophoretic mobility of the slow moving upper band now can be assumed equivalent to that of a tetramer. The mobility of the single band of HUM2 (Figure 1a, lanes 1−5) is equivalent to the upper band in K^+ (Figure 1b, lanes 2−6), confirms the identical molecularity of the structural species present, and can very well be assumed a tetramer.

Going a step further in this direction, we used a two-repeat *Tetrahymena* (TETRA) telomeric sequence of the length identical to that of the HUM2 sequence as a dimer control (*34*). In Figure 2a, the electrophoretic mobilities of HUM2 bands in Na^+ (lanes 4 and 5) could be inferred as tetrameric species, when compared with the fast moving bands of the

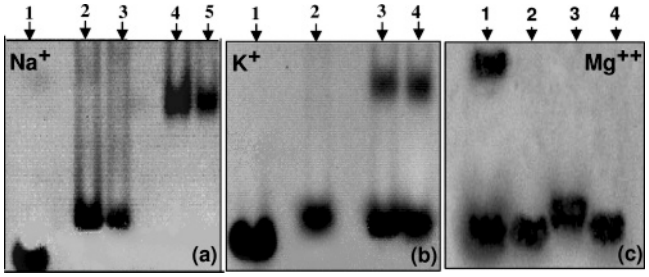

FIGURE 2: (a) 10% native PAGE mobility pattern of the oligonucleotide sequences. Lane 1: PAL (d-CTTGAGCTCAAG) used as the 12-mer duplex marker. Lanes 2 and 3: TETRA (d-TTGGGGTTGGGG) in 300 and 100 mM Na^+, respectively. Lanes 4 and 5: HUM2 (d-TTAGGGTTAGGG) in 300 and 100 mM Na^+, respectively. (b) 10% native PAGE mobility pattern of the oligonucleotide sequences. Lane 1: PAL (d-CTTGAGCTCAAG) used as the 12-mer duplex marker. Lane 2: TETRA (d-TTGGGGTTGGGG) in 100 mM K^+. Lanes 3 and 4: HUM2 (d-TTAGGGTTAGGG) in 300 and 100 mM K^+, respectively. (c) 10% native PAGE mobility pattern of the oligonucleotide sequences in 10 mM Mg^{2+}. Lane 1: PAL d-(CTTGAGCTCAAG) used as the 12-mer duplex marker + 60-mer single strand marker, M-60. Lane 2: HUM-2C (d-TTTGGGTTTGGG). Lane 3: HUM2 (d-TTAGGGTTAGGG). Lane 4: TETRA (d-TTGGGGTTGGGG).

TETRA dimeric control (lanes 2 and 3) and PAL (lane 1). It is clear that for both of the oligomers, the band intensity increases with the increase of Na^+ concentration, that is, the bands in 300 mM Na^+ (lanes 2 and 4) are more intense than the bands at 100 mM Na^+ (lanes 3 and 5). Similarly, in Figure 2b, where the HUM2 in K^+ showed two distinct bands (lanes 3 and 4) as found in Figure 1b, the fast-moving lower band of HUM2 was found to be migrating in a manner equivalent to that of the dimer control (Figure 2b, lane 2) and PAL (Figure 2b, lane 1). This observation indicated the molecularity of the two structural species formed by HUM2 in K^+. Accordingly, the species corresponding to the upper band is a tetramer (four-stranded), whereas the lower band represents the dimeric structure involving two strands. At this point, it is also important to mention that the dimeric species present in the lower band of the gel (Figures 1b and 2b) could be two types of quadruplexes (antiparallel or parallel) or may be a population of both conformations. This might be the reason for the more intensity of the lower bands in comparison to that of the upper one. Moreover, the comparative low intensities of the higher band could be due to the low population of tetramer species at the oligomer concentration used in the gel.

We also performed gel studies to obtain information on the structure through the molecularity status of the double repeat of the human telomere (HUM2) sequence in the presence of Mg^{2+}. The analysis of HUM2 in native PAGE containing 10 mM $MgCl_2$ depicted in Figure 2c confirmed that it exists as a dimer, migrating equivalent to that of the 12-bp duplex (PAL) size marker. Also shown are the dimers of well documented *Tetrahymena* double repeat and of the mutated HUM2 used as controls. Thus, it is clear that in Mg^{2+}, the quadruplex is dimeric in nature.

Thus, the altered gel mobility of the structural species of HUM2 during electrophoresis here, in non-denaturating 10% polyacrylamide gel in the presence of Na^+ and K^+ ions, brought evidence for the existence of dimeric and tetrameric quadruplex species of different geometries. Generation of a tetramer quadruplex structure in monovalent cation by the

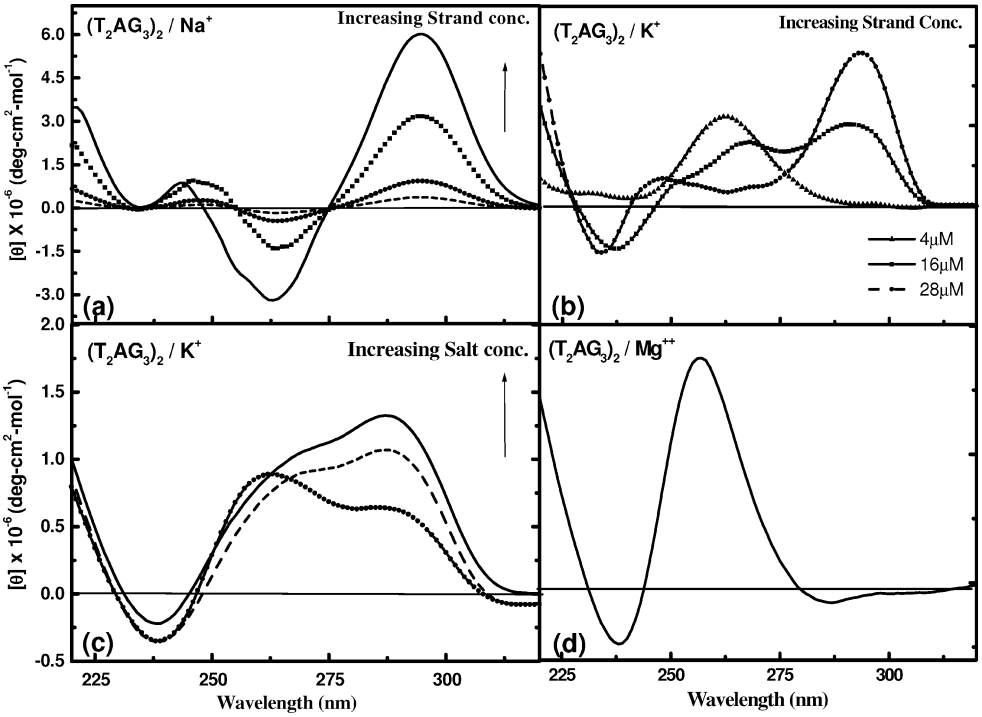

FIGURE 3: Circular dichroism spectra of HUM2 in 20 mM sodium cacodylate buffer (pH 7.4) and 0.1 mM EDTA (a) at strand concentrations of 4 μM (---), 8 μM (•••), 12 μM (■■■), and 16 μM (—) in 100 mM NaCl; (b) at strand concentrations of 4 μM (▲▲▲), 16 μM (■■■), and 28 μM (•••) in 100 mM KCl; (c) at salt concentrations of 100 mM (•••), 500 mM (----), and 1000 mM (—) KCl; and (d). at 10 mM $MgCl_2$.

double repeat of the human telomere sequence has emerged as an interesting finding. Moreover, an independent gel-shift study on the same sequence in K^+ has well illustrated the induction of dimeric and tetrameric quadruplex species by the ligand PIPER (*38*). However, the tetramer was not characterized in structural terms, that is, parallel and antiparallel quadruplex. In a more recent study on telomere oligonucleotides, ^{125}I radioprobing data has demonstrated that antiparallel conformation was present in K^+ and Na^+ solutions. The preferable conformation of d-(AGGG(TTAGGG)$_3$ in the Na^+ containing solution was a basket-type antiparallel quadruplex, whereas the presence of K^+ favored the chair-type antiparallel quadruplex (*39*). Interestingly, an almost identical repeat with guanines substituted with inosines shows parallel arrangements of strands in K^+ and mixed parallel and antiparallel conformations in Na^+ (*40*). A study conducted more than a decade ago on the Na^+ and K^+ complexes of d-(TTTTGGGG) and d-(TTTTGGGGT), also elegantly attempted the question of the possibility of an antiparallel tetramer in Na^+. Because d-(TTTTGGGG) generated higher order products in K^+ but not in Na^+, the author suspected that the tetrameric structure of strands could be antiparallel in Na^+ but parallel in K^+. Furthermore, the use of UV cross-linking established the parallel-stranded quadruplex structure (*41*). Our conclusion on the formation of an antiparallel tetramer structure by HUM2 sequence is based on a clear correlation between non-denaturating PAGE, CD, UV-melting experiments, and Gel-exclusion chromatography and is discussed in following sections.

Correlation with CD Spectral Characteristics. The circular dichroism spectroscopic method has been particularly proved to be informative in elucidating the conformation of nucleic acid secondary structures. Extensive CD studies have revealed that G-quartet structures have a characteristic CD profile, and therefore, it has proved to be a very sensitive technique for probing the structure of telomeric models (*21*, *41*, *42*). Though recent reports (*43*, *44*) have suggested the need for caution in the interpretation of CD spectra relating quadruplex properties, in most cases, they have proved to be a useful guide (*45*).

We asked, what types of structures of HUM2 are involved in tetramer and dimer formation? Knowledge of the type of DNA quadruplex involved (parallel or antiparallel), combined with gel results, would in turn provide information on the number of strands involved in G-tetrad formation.

Because an alkali metal ion can determine the type of G-quadruplex formed, HUM2 samples in the range of 4−16 μM were incubated in 100 mM NaCl, 20 mM sodium cacodylate buffer (pH 7.4), and 0.1 mM EDTA. CD spectra of HUM2 at strand concentrations of 4, 8, 12, and 16 μM in Na^+ at 20 °C are displayed in Figure 3a. The sequence exhibited a strong positive band at 295 nm and a negative band at 263 nm, identical to the CD spectra attributed to antiparallel G-quadruplex assemblies (*16*, *21*). Increase in the CD amplitude at 295 nm with ascending concentration of HUM2 oligonucleotide signifies the increased population of antiparallel species.

Similarly, the CD spectra of HUM2 in 100 mM KCl, at the increasing oligomer concentrations of 4, 16, and 28 μM, were recorded and are displayed in Figure 3b. Out of the three concentrations used, to our surprise, at 4 μM concentration of HUM2 a single positive peak at 265 nm corresponding to the presence of parallel quadruplex species was observed, whereas at the intermediate concentration (16 μM), consistent to our earlier observation, two positive bands observed at 265 and 295 nm highlighted the presence of parallel and antiparallel species. Finally, at 28 μM strand concentration of HUM2, the 265 nm peak disappeared, and

only the 295 nM positive peak survived, indicating the presence of only antiparallel quadruplexes. Thus, as expected, HUM2 showed oligomer concentration dependence, displaying an increasing intensity of the 295 peak accompanied by a decreasing intensity for the 265 nm peak with every rise in oligomer concentration. On the basis of the correlation of CD results with gel studies, the two quadruplex species appearing in the 100 mM K^+ at moderate strand concentrations (10−16 μM) were interpreted in terms of different molecularities, that is, two- and four-stranded structures. The observed concentration dependence in the CD experiment, complying well with the simple mass action considerations, conclusively favored the four-stranded form.

The CD spectra of HUM2 under identical buffer conditions but as a function of salt concentrations (100, 500, and 1000 mM K^+) are displayed in Figure 3c. The CD spectra in K^+ showed a negative band at 240 nm followed by two positive bands at 265 and 295 nm. Because a positive band centered at 260−265 nm and a negative band at 240 nm are characteristic of parallel quadruplex structure (*41*, *42*), the presence of two positive bands at characteristic wavelengths indicates a mixture of parallel and antiparallel quadruplex species. With an increase in K^+ concentration, the ellipticity of the 295 nm positive peak increases, whereas 260−265 nm peaks remains more or less same. This signifies that increase in K^+ concentration facilitates the formation of the tetrameric antiparallel quadruplex. Surprisingly, even 1 M KCl could not shift the equilibrium completely toward one form of quadruplex. Furthermore, when the concentration of Na^+ was increased from 100 mM to 1 M, the CD spectra exhibited the signatures of only antiparallel quadruplex forms; with every increment of salt concentration, the 295 nm positive peak showed an increase in molar ellipticity (data not shown). Our observation shows that K^+ favors the formation of both types of quadruplexes, whereas Na^+, favors only the antiparallel species.

Furthermore, we also studied the status of HUM2 in the presence of magnesium. To our surprise, the CD spectrum recorded at 10 mM $MgCl_2$ displayed a positive peak at 265 nm and negative peak at 240 nm, which was diagnostic of parallel quadruplex forms (Figure 3d). No signatures of the antiparallel form of the quadruplex were detected. Thus, it is clear that in Mg^{2+}, the dimeric quadruplex (concluded from PAGE, Figure 2c) is found to be a parallel-stranded quadruplex and should possibly adopt a propeller-type quadruplex structure, as reported by X-ray studies (*23*). Mg^{2+} has been shown to induce structural transition from antiparallel to parallel G-quartet by destabilizing the former (*75*). The quantitative effect of the divalent cation on G-quartet structures, however, is not clear.

It is an accepted fact that 1 M Na^+ or K^+ mimics the generic activity of 10 mM Mg^{2+} as a backbone counterion. However, even after increasing the concentrations of the monovalent cations up to 1 M, the Na^+ and K^+ ions did not behave equivalently, and neither ion could accurately predict the structure in Mg^{2+}.

It is worth mentioning here that the antiparallel quadruplexes studied so far were reported either to be monomolecular or bimolecular, whereas the parallel quadruplex were found to be tetramolecular (*15*, *18*). It is only recently that parallel quadruplexes were also seen as dimeric structures (*23*, *26*). Comparing our gel-shift analysis with CD results, it is now possible to correlate a particular CD characteristic with a particular type of DNA quadruplex. Accordingly, we conclude that Na^+ induces a tetrameric antiparallel quadruplex in HUM2, whereas K^+ stabilizes the dimeric parallel and tetrameric antiparallel structures. Consistent with our observation, a recent study of various vertebrate telomeric repeats using CD and NMR has concluded that in the case of TTAGGGTTAGGG, a high concentration (140 mM) of sodium favors antiparallel quadruplex structures (*46*). However, the main conclusion of this report that propeller (parallel) quadruplex structures are favored in the presence of high concentrations of potassium seems to differ with our results in potassium. The reason for this discrepancy is our experimental conditions, which differ in terms of the salt concentrations used. The concentrations of K^+ used by Rujan et al. were 0, 1, 10, or 100 mM KCl, whereas our experiments used a range of 50−1000 mM KCl. Interestingly, the nature of the CD spectra of TTAGGGTTAGGG at 100 mM KCl reported by Rujan et al. (Figure 3, middle panel in ref *46*) is in agreement with the one recorded by us under identical conditions (Figure 3c). The spectrum displays a major positive peak at 260−265 nm, followed by a minor positive peak at 295 nm, indicating the simultaneous presence of parallel and antiparallel quadruplex structures. Because our gel as well as CD experiments showed an increase in antiparallel G-quadruplex species at a higher concentration range, it is likely that the conclusion of Rujan et al. is valid for a narrow range of potassium (i.e., <100 mM KCl), where the major species are the parallel (propeller) quadruplex.

Differential Thermal Stability of the Quadruplexes of HUM2. Representative thermal melting profiles of HUM2 (10 μM strand concentration) in 20 mM sodium cacodylate buffer (pH 7.4) containing either 100 mM NaCl or KCl are shown in Figure 4. The melting curve of HUM2 in the presence of 100 mM NaCl was found to be monophasic, indicating the melting of one type of the self-associated form of the oligonucleotide. In view of the conclusion drawn from the gel-shift study and CD experiments clearly interpreted in terms of the tetrameric antiparallel quadruplex form, the observed melting behavior of HUM2 (Figure 4a) in Na^+ in a monophasic manner was expected. The calculated melting temperature of 55 °C, could be the T_M of the proposed tetrameric form of antiparallel G-quadruplex. On the contrary, in the presence of 100 mM KCl, an apparently broad and biphasic melting profile was obtained (Figure 4b), indicating the existence of two species, and each showed distinct quadruplex melting transitions. A lower temperature transition with a T_M value of 58 °C is followed by a higher temperature transition with a T_M value of 80 °C. Keeping in mind that K^+ induced inter/intramolecular G-quartets are more stable than their Na^+ counterparts (*14*), it can be correlated that the lower temperature transition with a T_M value of 58 °C corresponds to the melting of the antiparallel quadruplex species, whereas the higher temperature transition of the biphasic curve presumably is the melting of the stable dimeric parallel quadruplex species. This sequential melting can be explained in terms of the entropy penalty of complex formation and is consistent with the conventional insight that the tetramer should form at lower temperature because of its higher unfavorable entropic contribution. This perception has already been accepted and well documented (*47*).

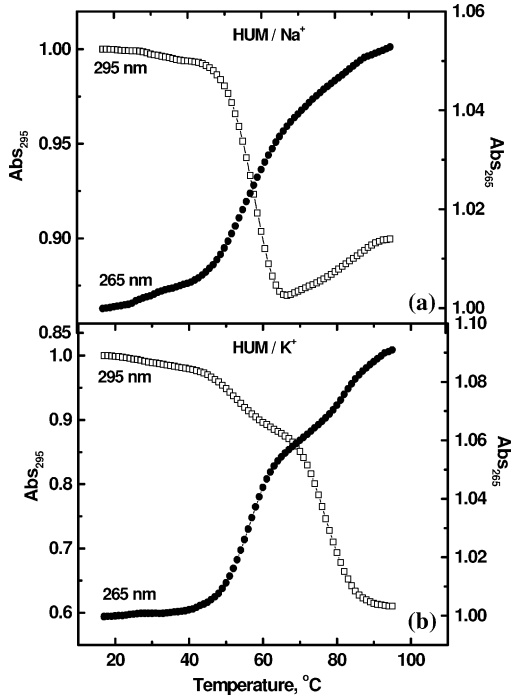

FIGURE 4: Thermal denaturation profiles of HUM2 (d-TTAGGGTTAGGG) in 20 mM sodium cacodylate buffer (pH 7.4) and 0.1 mM EDTA containing 100 mM NaCl (a) and 100 mM KCl (b) monitored at 265 nm (•••) and 295 nm (□□□).

Quadruplex melting monitored at 265 nm (A_{265}) shows a sigmoidal (hyperchromic) curve, whereas monitoring the absorbance change with temperature at 295 nm (A_{295}) displays a characteristic inverse sigmoidal (hypochromic) curve (*31*). HUM2 at 265 nm shows a monophasic sigmoidal curve while at 295 nm in Na^+ containing solution exhibited an inverse monophasic sigmoidal curve (Figure 4a). Analysis of the 265 and 295 nm profiles led to identical melting temperatures, the T_M value being 55 °C. An inverse sigmoidal curve obtained in UV-melting experiments at 295 nm again supports the presence of the G-quartet-containing structures adopted by HUM2. Interestingly, similar results were obtained when the thermal denaturation on HUM2 was performed in the presence of K^+. Likewise, monitoring absorbance at 295 nm produced a biphasic inverse melting profile, confirming the existence of two quadruplex species. Recently, d-(GGGT) has been shown to form an interlocked quadruplex dimer, whereas UV-melting studies indicated the existence of two structures quite distinguished by low-T_M and high-T_M species. CD and NMR studies concluded that they were parallel quadruplexes of distinct molecularity (*48*).

Numerous studies on telomeric sequences have described the differential stabilities of G-quadruplex structures in terms of the effect of counterions: the quartet structures are sensitive to monovalent cations (*13*, *14*). Both Na^+ and K^+ are known to effectively stabilize many quadruplex structures where K^+ is more potent than Na^+. It has been proposed that a sodium−potassium conformational switch regulates the structure opted by the tetraplex forming oligonucleotides (*49*). There is also some variability in the literature regarding the effect of K^+ and Na^+ on the nature of the quadruplexes they stabilize. An opposite behavior to the earlier report (*49*) has been illustrated for the sequence d-(GGGTTTTGGG) and d-(GGGGTTTTGGGG), where Na^+ only stabilizes the hairpin dimer quadruplex, whereas both hairpin dimer and linear 4-stranded quadruplexes are formed in the presence of K^+ (*50*). Such apparent discrepancies or sequence-dependent differences have also been reported for other metals (*13*, *51*). On the basis of gel, NMR, and CD results, it was found that a change in counterions from Na^+ to K^+ specifically induced conformational transitions in d-$(TTGGGG)_4$, resulting in a change from the intramolecular to apparent multistranded structures accompanied by an enhancement of melting temperature by >25 °C (*42*).

In a separate study (*21*), the effect of K^+ on the structure and stability of human telomeric oligonucleotides has shown that the stability of G-quartet structure depends on the number of repeats and cations. The oligonucleotide d-$(TTAGGG)_4$, which forms an antiparallel intramolecular G-quartet structure melts at 49 °C in 70 mM NaCl, whereas its K^+ induced structure melts at 63 °C. Similarly, under identical conditions, the 1.5 copy sequence d-(GGGTTAGGG) melts at 31 and 42 °C in 70 mM NaCl and KCl, respectively. CD results confirmed the presence of parallel and antiparallel quadruplex species in K^+; however it was not specified as to which structural type of the quadruplex corresponded to the reported T_M value. The higher T_M value for the HUM2 sequence obtained in the presence of K^+ relative to the value in Na^+ is in agreement with these reports. The structural species corresponding to the higher temperature transition (T_M 80 °C) could be the parallel quadruplex dimer form, meticulously identified by X-ray and NMR methods (*23*, *26*).

Separation of Structures Adopted by HUM2, Using Gel Filtration. Gel filtration or gel-permeation chromatography (GPC) is based on simple logics of size-exclusion chromatography (SEC), where biomolecules are separated on the basis of their molecular weights. Molecules that are smaller than the pore size of the gel can enter the particles and therefore have a longer path and longer transit time than larger molecules that cannot enter the particles; hence, larger molecules come out first.

For the separation of two different oligomeric structural species (tetramer and dimer) formed by HUM2 sequence, gel filtration was performed. A concentrated sample of HUM2 (200 μM) containing 100 mM KCl was allowed to pass through an equilibrated size-exclusion column of Bio-Gel P-10 matrix, and chromatography was performed at a low flow rate for sometime, collecting fractions of 50−60 μL each. The absorbance of fractions was measured at 260 nm and was finally plotted against the elution volume (in μL) of the oligomer (Figure 5a). As expected, the elution profile showed two prominent peaks. The major, first peak (eluted first) showing higher absorbance value corresponds to the tetrameric form of the quadruplex, whereas the second peak (eluted later) with comparatively less absorbance was interpreted as the dimeric (quadruplex) form of the HUM2. The molecularity of the structural forms was further confirmed when the two eluted fractions were run on a non-denaturating gel. Their gel-mobility pattern when compared with that of the control size markers confirmed the molecularity of the structures. The slow-moving band of the first eluted fraction (Figure 5b, lane 1) compared with the mobility of a 60-base oligonucleotide marker (Figure 5b, lane 2) was suggested to be the tetrameric quadruplex (48 base), whereas the fast-moving band of the second eluted fraction (Figure 5b, Lane 6) migrating equivalent to a duplex marker PAL (24 base) (Figure 5b, lane 4) and the dimeric control HUM2C

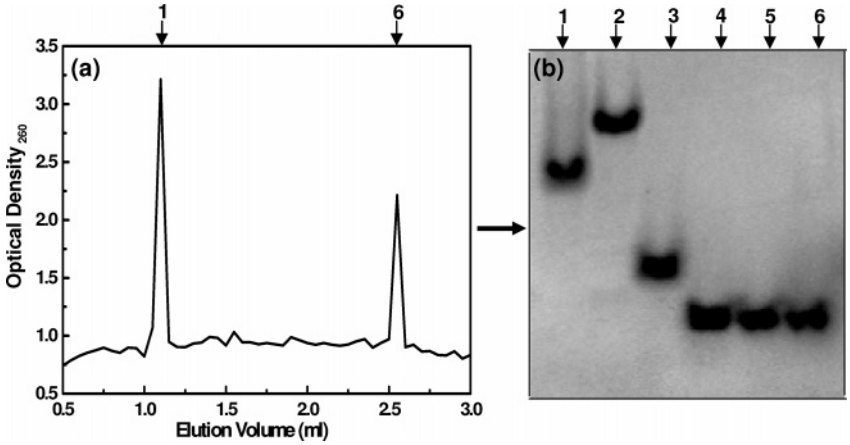

FIGURE 5: (a) Gel-filtration chromatograph of HUM2 (d-TTAGGGTTAGGG) on a Biogel P-10 column with elution buffer being 20 mM Na cacodylate containing 100 mM K^+ and 0.1 mM EDTA. The fractions corresponding to the peaks designated as 1 and 6 were run in lanes 1 and 6 of the gel in Figure 5b. (b) Ten percent native PAGE mobility pattern of the oligonucleotide sequences. Lane 1: first elution, HUM2 (d-TTAGGGTTAGGG) 100 mM K^+ (TETRAMER). Lane 2: 60-mer single strand marker, M-60. Lane 3: 35-mer single strand, marker M-35. Lane 4: PAL (d-CTTGAGCTCAAG) used as the 12-mer duplex marker. Lane 5: HUM-2C (d-TTTGGGTTTGGG) in the presence of 100 mM K^+. Lane 6: second elution, HUM2 (d-TTAGGGTTAGGG) 100 mM K^+ (DIMER).

(Figure 5b, Lane 5) was unambiguously attributed to the dimeric quadruplex (24 base). Thus, gel filtration gave us an analytical evaluation of the presence of dimeric and tetrameric forms of HUM2 oligonucleotide in the presence of K^+.

Possible Structural Model for Tetrameric Antiparallel Quadruplex. In order to rationalize the results presented in this article, we propose a structural model for the antiparallel quadruplex comprising four strands formed by the double repeat of human telomere d-(TTAGGGTTAGGG). The proposed model predicts the type of molecular architecture that should occur in a manner consistent with structural and kinetic considerations of G-quadruplexes (*13*, *14*). Accordingly, a quadruplex structure made up of four strands of d-(TTAGGGTTAGGG) (HUM2) with antiparallel orientation acquiring an anti-anti−syn-syn conformation of the guanine base is proposed. Such an arrangement was confirmed for *Oxytricha* sequence d-(GGGGTTTTGGGG) by NMR (*52*) showing the adjacent strands alternatively parallel and antiparallel with a pattern of syn-syn−anti-anti glycosidic angles for each quartet. The interesting part of the proposed model structure is that the intervening -TTA- bases that occur between G-tetrad-forming guanines might form two Watson−Crick base pairs linking two strands of opposite polarity (Figure 6a iii and 6b). These two Watson−Crick AT base pairs can only be visualized if HUM2 adopts a structure with adjacent strands being alternatively parallel and antiparallel. For facilitation of hydrogen bonding between the complementary bases in the intervening region of G-tracts, one of the strands should be displaced by three bases toward the 5′- side. Thus in total, two of the four strands with identical polarity will be slipped by three bases, leaving the antiparallel quadruplex with protruding 5′-ends of two parallel strands. The central thymine of the −TTA- loop segment on each of the four strands of the tetramer might possibly bulge out of the helix axis, in turn to facilitate better stacking between AT/TA base pairs. Moreover, the possibility of a T-tetrad in a parallel-stranded DNA quadruplex has already been reported by NMR (*53*), but we are not sure at this stage whether any such arrangement for the central thymines can take place in the proposed antiparallel tetramer of the HUM2 sequence. Although we do not present direct evidence that demonstrates a specific strand polarity for the four-stranded complex, the possibility of the tetraplex containing strands with opposite polarity has not been denied in literature. Such possibilities are depicted in Figure 6a (ii, iii, and iv).

Furthermore, to validate the proposed model, we have also mutated HUM2 by substituting the central -A- with -T-, thus leaving hardly any possibility for Watson−Crick hydrogen bonding as suggested (Figure 6b). Comparing the mutated sequence d-TTTGGGTTTGGG (HUM2C) with the original d-TTAGGGTTAGGG (HUM2), a substantial difference was observed in their gel mobilities in the presence of K^+ (Figure 5b). Accordingly, HUM2C (Figure 5b, lane 5) moves with PAL (lane 4), confirming its dimeric status, which in turn is equivalent to the dimer of HUM2 (Figure 5b, lane 6, second eluted fraction of gel filtration), whereas the HUM2 tetramer (Figure 5b, lane 1, eluted first fraction of gel filtration) migrated above the marker M-35 (Figure 5b, lane 3), reflecting a mobility equivalent to that of the tetrameric species. It can be assumed that in the absence of the possibility of A−T base-pairing between HUM2C strands, it adopts a folded dimeric structure such as that of the *Tetrahymena* double repeat (d-TTGGGGTTGGGG, Figure 2a and b). Thus, this control experiment is in accord with our interpretation of an antiparallel four-stranded model and establishes up to some extent that two A−T base pairs are possibly involved in the tetrameric structure adopted by HUM2.

The model also explains that though the tetramer has six G-quartets in comparison to those in the dimer (with only three G-quartets), it is less thermally stable than the latter. This difference can be attributed to the fact that the proposed tetrameric antiparallel quadruplex (four-stranded) has more open and fraying ends than the dimer, which might lead to the early melting (destabilization) of the tetrameric structure. Another reason might be that though there are six G-quartets and four base pairs, the structure is not a continuous one. The upper set of three G-quartets in the proposed antiparallel tetraplex is separated from the lower set by three bases upsetting the stacking interactions between the six G-quartets. Similarly, each set of the two A−T base-pairs is also

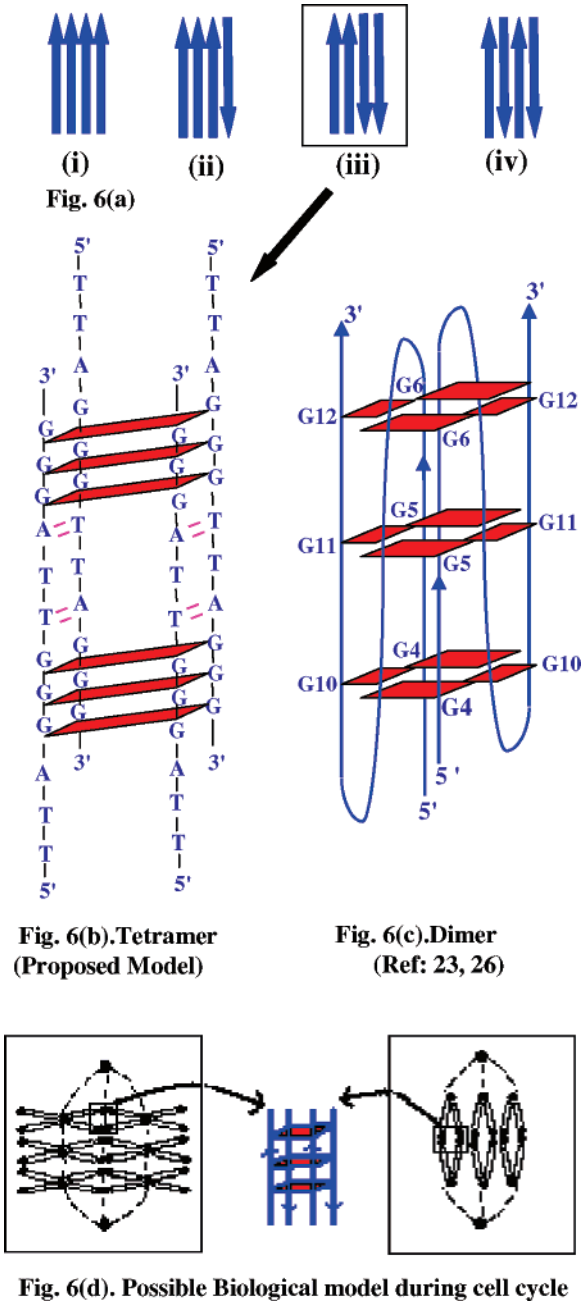

Fig. 6(d). Possible Biological model during cell cycle

FIGURE 6: Proposed models for the antiparallel (tetramer) quadruplex.

separated from the other by an internal bulge of four Ts, thus affecting the overall stability of the tetramer. On the contrary, the parallel quadruplex formed by the dimeric association of two HUM2 strands may be stabilized by three well stacked G-quartets and by the Watson−Crick, A−T base pairing within the -TTA- loop of each strand, making it a compact and more stable structure than the antiparallel tetramer; such a possibility has been suggested (*21*). Thus, the inclusion of A and T into guanine-rich sequences gives a possibility of structures partially stabilized by Watson−Crick base pairing. The presence of potential Watson−Crick base pairs within a guanine-rich sequence has a profound effect on the final structure of a folded quadruplex, and such a possibility has been suggested (*21*).

With the proposed model, the possibility of G-wire formation through the engagement of TTA arms at the 5′-end on both sides of the quadruplex in interactions through Watson−Crick A−T base pairing cannot be ruled out. Such assemblies or higher molecular weight species were not observed in the native or denaturating gels.

At this point, it is important to disscuss some other possibilities of tetrameric structure. The HUM2 sequence may form a dimer of dimers. Such a structure would have the terminal TTA sequences simply oriented away from the stack, as has been observed in some X-ray structures (*23*). Accordingly, two antiparallel hairpin dimers may dimerize head to head with three G-tetrads stacking on top of each other, resulting in a dimer of dimers with a continuous stack of six G-tetrads. Also, there might exist another possibility of two hairpin dimeric (antiparallel) G-quadruplexes zipping one another through the 5′-TTA overhangs with the formation of T−A−T−A tetrads.

Although we cannot absolutely exclude the possibility of the two hairpin dimeric (antiparallel) G-quadruplex, we have discussed our view points of analysis and maintain that the antiparallel quadruplex population identified in PAGE experiments is tetrameric in nature.

As apparent from the CD spectra, in the presence of sodium, only the antiparallel quadruplexes are formed, and the molecularity of the same was suggested to be tetrameric through gel assays. The possibility of a tetramer existing as a dimer of dimers can be ruled out on the basis of the following analysis. First, had it been a dimer of dimers, then owing to an established equilibrium between its unit dimer and dimer of dimer species there should be at least some dimer visible as one more band in gels containing sodium, which would move in a manner equivalent to that of PAL (the 12-bp duplex). No additional band was observed in sodium-containing native gels. Second, the UV thermal denaturation profiles were always found to be monophasic in the presence of sodium. A dimer of dimers would melt in two stages, with a lower transition reflecting in the separation of two dimers followed by a higher temperature transition corresponding to disordering of the unit dimer. No such biphasic melting curve was obtained in sodium. However, we observed biphasic curves in the presence of potassium, interpreted correctly as the lower temperature melting of the antiparallel tetramer and higher temperature disordering of the parallel dimer (propeller type) quadruplex species.

At this point, it is important to highlight the recently emerged structures of two-repeat human telomere segment d-(TAGGGTTAGGGT) solved independently in crystal (*23*) and in solution (*26*) containing K^+ as the counterion. The X-ray study defined a novel dimeric G-quadruplex, with all of the strands parallel with guanine in anti conformation and three linking trinucleotide loops positioned on the exterior of the quadruplex core in a propeller-like arrangement. Alternatively, the NMR structure of the identical sequence detected the coexisting dimeric parallel (propeller-like) and antiparallel quadruplex species with different thermodynamic properties and different kinetics of folding and unfolding (Figure 6c). A very recent platinum cross-linking study of the human telomere sequence $AG_3(T_2AG_3)_3$ and $(T_2AG_3)_4$ also suggested that the antiparallel structure exists in the presence of both Na^+ and K^+ ions (*35*). The literature is rich in reports about different folding patterns of the G-rich telomere strand into different types of G-tetraplexes. To date, the exact structure of human telomeric G-quadruplexes in

K^+ solution is extremely controversial. The truncated or the full four-repeat sequence has been extensively studied by various biophysical methods, including CD, NMR, and X-ray (*23*, *26*, *55*−*57*). The recent models proposed for d-AGGG-$(TTAGGG)_3$ in K^+ solution include a mixture of mixed parallel/antiparallel and chair-type G-quadruplex (*54*), whereas d-AAAGGG$(TTAGGG)_3$AA was found to form a hybrid-type intramolecular G-quadruplex structure with mixed parallel/antiparallel strands (*57*). Addition of K^+ readily converts the Na^+-form conformation to the K^+-form hybrid-type G-quadruplex. Furthermore, secondary structures of the fragments $G_3(TTAG_3)n$, where $n = 1-16$ have been studied, and it was found that folding of these fragments into tetraplexes depends on the number of $TTAG_3$ repeats. The suggested topologies along with the antiparallel and parallel bimolecular tetraplexes include a tetraplex consisting of three parallel chains and one antiparallel chain (*55*). This unique structure of the four-repeat human telomeric sequence with $(3 + 1)$ topology has recently been investigated by NMR in K^+ solution (*56*).

Biological Relevance of the Proposed Tetrameric Antiparallel Quadruplex Structure. G-quadruplexes formed by the human telomeric sequences have received much interest over more than a decade because of their well illustrated polymorphic behavior, structural distinctiveness, and their biological significance. The 3′-strand of the telomere DNA consisting of tandemly repeated sequence motifs that typically contain clusters of 3 Gs extends over the complementary strand as an overhang. Our sequence of interest has been such an overhang with a double repeat of TTAGGG. The same is not only restricted to humans but also present in eukaryotes, protozoan, slime moulds, filamentous fungi, and other vertebrates. The 12-nt long sequence TTAGGGTTAGGG is shown to form an antiparallel G-tetraplex in the presence of sodium, and this information provides a model for the physiological structural status of the single-stranded G-rich overhangs protruding from the genomic duplex at telomeres.

Despite unifying origin, the most intriguing aspect of G-quadruplexes is their extensive polymorphisms, that is, they can adopt inter and intramolecularly folded structures. G-rich telomeric single strands can fold into DNA quadruplex structures, which may inhibit telomerase activity by inaccessibility of the telomeric primers to telomerase (*59*). There is no report that might have concluded that there are biological roles for DNA G-quadruplexes built on stacked guanine tetrads, but recent discoveries of manifold human proteins that recognize them *in-vivo* strongly suggest that this is the case (*1*, *60*, *61*). The most direct evidence for G-quadruplex DNA existing in cells is that antibodies raised against G-quadruplex DNA label the macronuclei of a ciliate *Stylonychia lemnae* (*61*). A recent report by Chang et al. also verifies the existence of the quadruplex structure at telomere proximal regions in metaphase chromosomes (*62*). Whereas there are proteins that recognize G-quadruplexes and promote their formation, there also exists a class of proteins that catalyze their disintegration (*59*, *63*).

Telomere-binding proteins might show specificity or selectivity for structural features rather than for a particular sequence and thus would preferentially bind to one form of quadruplex (parallel/antiparallel) of tetrameric topology rather than an intramolecularly folded or dimeric G-quadruplexe. Specificity in protein binding to quadruplex structures in comparison to single- and double-stranded DNA raises the possibility that such proteins could serve not only to recognize preformed quadruplexes but also to promote the formation of quadruplexes *in vivo*. Generated *in vitro*, one of the single-chain antibody fragments (scFv) Sty3, with a high affinity for the parallel-stranded G-quadruplex, could discriminate between parallel or antiparallel quadruplex species formed by the same guanine 3′-overhangs sequence of a ciliate telomere (*61*). Telomeres are now shown to have a role in chromosome separation during mitosis. A telomerase template mutation caused a physical block in anaphase chromosome separation (*64*). The role of telomere cap structure in meiosis has also been suggested in budding yeast (*65*).

On the basis of existing knowledge and conclusions drawn from well documented works, we have attempted to reveal the biological relevance of our proposed model. The model depicted in Figure 6d for antiparallel G-tetraplex structure could be created by the association of 3′-overhangs of the telomeres of two chromosomes (four sister chromatids) aligned and brought together on the equatorial plane of the spindle during metaphase and similarly between the 3′-overhangs of two sister chromatids (V shaped) placed opposite each other during anaphase. In eukaryotic cells, the chromosomal protein cohesin holds the sister chromatids together until they separate into daughter cells during mitosis. This could facilitate the 3′-overhangs to associate them end to end (antiparallel) via their telomeres. Cohesin sites are highly conserved in mitosis and meiosis, suggesting that chromosomes share a common underlying structure during developmental programs. The proposed structure has the possibility of Hoogsteen (G−G) and Watson−Crick (A−T) hydrogen bonds, representing the self-recognition and complementarity existing simultaneously. Although there seems to be a possibility that telomere end-binding proteins (TEBPs) may play an important role in telomere−telomere interactions and may control the formation of G-quadruplex DNA *in vivo* (*60*), the intrinsic property of self-association by guanine-rich sequences may be the prime driving force in the formation of the antiparallel G-tetraplex. The concept of self-recognition between single-stranded G-rich termini has been used to propose a general mechanism for the DNA-mediated association of chromatids (*34*, *66*). G-quadruplex structures must be dynamic and can be reformed whenever the cells need them. For example, Paeschke et al. have found out that G-quadruplex formation *in vivo* is regulated by the cell cycle-dependent phosphorylation of TEBPβ (*60*).

On similar lines, more than 15 years ago, Sen and Gilbert had proposed a scheme for the parallel-stranded G-tetraplex formed within the four homologous chromatids during meiosis. In the stages of prophase-I, the homologous chromosomes appear precisely aligned side by side, facilitating the association of telomeres through G4-DNA formation (*67*). Quite recently, Peter Baumann has also proposed a model for the association and dissociation of G-quadruplexes mediated by TEBPα and phosphorylation of TEBPβ, respectively. Shown is the possibility of a stable complex formed by G-rich overhangs from two or more telomeres, which may mediate chromosome associations (*68*).

The molecular nature of the very tip (G-rich 3′-overhang) of the telomeres is crucially important for carrying out

various cellular functions. They may function as anchors used in telomere−telomere associations and as antennas that detect ionic conditions that facilitate the quadruplex formation of a particular type. The strands could be used to sense appropriate changes at physiological conditions within the nucleus. The forms and stability of G-quartet structures can be modulated by the fluctuations in the Na^+/K^+ concentration in the cell and the number of such sequence repeats present at the 3′-overhang of the ends of chromosomes. Any change in K^+ concentration could alter the chromatin structure at telomeres by taking advantage of the unique sensitivity of quadruplex formation to Na^+ and K^+ concentrations (*13*). Direct evidence of *in vivo* chromosome association under the control of ratio of $[Na^+]$ and $[K^+]$ has not yet been obtained, but a postulated theory that the biological role of telomere tails may promote chromosome association (e.g., synapsis during meiosis) under the control of specific alkali metal ions does exist (*69*). A recent biophysical study on telomeric DNA highlights the possibility of external loop structures in the vertebrate telomeres under conditions of high potassium and low sodium concentrations found in nuclei (*46*). There are several proteins that bind with high affinity to G-quadruplex structures. Defects in these proteins can lead to errors in replication, transcription, and recombination as well as increases in the rate of tumor formation and aging (*3*, *70*, *71*). Interconvertible quadruplex structures in terms of molecularity or structural type might selectively be recognized by proteins or synthetic ligands. Our proposed model of the antiparallel guanine tetraplex could be a biologically relevant conformation of DNA. Such structures might be involved in the molecular mechanism by which sister chromatids cohere and separate in a timely manner in specific cell cycle stages.

CONCLUSIONS

In the present study, the polymorphic behavior of the double repeat of human telomere sequence HUM2 d-(TTAGGGTTAGGG) was investigated. Our analysis supports the formation of one and two structural species in presence of Na^+ and K^+, respectively. Using complementary techniques (gel electrophoresis, circular dichroism and UV melting, and gel-filtration chromatography), we analyzed the structural type and molecularity adopted by HUM2 sequence. Gel-shift assays revealed the presence of two bands with differential mobilities in the presence of K^+, whereas a single band was obtained in Na^+. Comparing the mobilities of oligomeric bands with suitable controls, we concluded that the presence of K^+ induces tetrameric and dimeric quadruplex structures, whereas Na^+ only generated tetramers. Separation of the oligomeric species through gel filtration further supported the presence of tetrameric and dimeric structures of HUM2.

In the CD analysis, the structural type of the G-quadruplex was determined with the standard information available in the literature about the CD signatures for the parallel and antiparallel quadruplexes. Correlation of gel assays and CD results demonstrated that the antiparallel quadruplex species is tetrameric, whereas the parallel one turned out to be dimeric.

On the basis of the correlation of gel-shift studies with CD, a model has been proposed for the tetrameric antiparallel quadruplex. There seems to be quite a possibility of two Watson−Crick base pairs being present between the intervening (-TTA-) sequences on the opposite strands, well accommodated between blocks of three tetrad stacks. The model could be a biologically relevant nucleic acid structure.

Extremely diverse conformations in human telomeric DNA expand the repertoire of G-quadruplex structures and may have implications for the proteins and drugs that recognize G-rich sequences (*58*). Our finding of a tetramolecular antiparallel quadruplex formed by the full double repeat of human telomere d-(TTAGGGTTAGGG) seems to be one of the growing indications about the polymorphic temperament of G-quadruplexes. In view of our results, it is intriguing to note the reported coexistence of parallel and antiparallel dimeric forms by an almost identical sequence d-(TAGGGTTAGGGT), differing in one base from HUM2 (T at 3′-end) by NMR. The dimeric antiparallel quadruplex species of d-(TAGGGTTAGGGT) identified by NMR (*26*) in the presence of K^+ was not detected in X-ray analysis (*23*). Similarly, it seems that one base difference in the sequence studied here and earlier by NMR and X-ray methods (*26*, *23*) could account for the inability of the latter sequence to form the antiparallel tetrameric quadruplex. Also the difference in oligomeric concentrations used during NMR, X-ray CD, UV-melting, or gel studies might be one of the reasons for the structural heterogeneity of the various structures formed by the studied guanine-rich sequences. Markedly, there seems to be a delicate balance of experimental and solution factors, such as the effective cation concentration, oligomer concentration, and the necessity to prepare a single species for structure determination, mode of crystallization, and so forth. In an independent study, we found it difficult to solve the NMR structure of a DNA hairpin conformation, which is demonstrated to exist only in the range of 10−40 μM oligomer concentration, above which it converts into a bulge duplex form (*72*, *73*). Furthermore, a very recent study from the Shafer group (*36*) using CD and gel studies has added to the structure of the human telomeric sequence revealed by X-ray studies (*23*) and showed that the same sequence can adopt an antiparallel rather than parallel G-quadruplex structure. A recent report from the Chaires group (*74*) also confirmed the intramolecular antiparallel quadruplex structure of d-$AG_3(T_2AG_3)_3$ in the presence of Na^+ and has also shown that a much greater conformational heterogeneity exists in solution than in crystal. There has been much debate as to the exact conformation of the quadruplexes under physiological conditions, and it has become increasingly apparent that the human quadruplex may be more structurally heterogeneous than that of other species.

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REFERENCES

1. Blackburn, E. H. (2001) Switching and signaling at the telomere, *Cell 106*, 661−673.
2. Rhodes, D., Fairall, L., Simonsson, T., Court, R., and Chapman, L. (2002) Telomere architecture, *EMBO Rep. 3*, 1139−1145.

3. Cech, T. R. (2004) Beginning to understand the end of the chromosome, *Cell 116*, 273−279.
4. Sundquist, W. I. (1991) *Nucleic Acids and Molecular Biology* (Eckstein, F., and Lilley, D.M.J., Eds) Vol. 5, pp 1−24, Springer-Verlag, Berlin, Germany.
5. Simonsson, T., Pecinka, P., and Kubista, M. (1998) DNA tetraplex formation in the control region of *c-myc*, *Nucleic Acids Res. 26*, 1167−1172.
6. Wells, R.D., and Warren, S.T. (1998) *Genetic Instabilities and Hereditary Neurological Diseases*, Academic Press, New York.
7. Herrick, G., Cartinhour, S., Dawson, D., Ang, D., Sheets, R., Lee, A., and Williams, K. (1985) Mobile elements bounded by C_4A_4 telomeric repeats in *Oxytricha fallax*, *Cell 43*, 759−768.
8. Sen, D., and Gilbert, W. (1988) Formation of parallel four stranded complexes by guanine rich motifs in DNA and its implications for meiosis, *Nature 334*, 364−366.
9. Kelland, L. R. (2000) Telomere inhibitors: targeting the vulnerable end of cancer ? *Anti-Cancer Drugs 11*, 503−513.
10. Oganesian, L., and Bryan, T. M. (2007) Physiological relevance of telomeric G-quadruplex formation: a potential drug target, *BioEssays*. *29*, 155−165.
11. Rezler, E. M., Bearss, D. J., and Hurley, L. H. (2002) Telomeres and telomerase as drug targets, *Curr. Opin. Pharmacol*. *2*, 415−423.
12. Davis, J. T. (2004) G-quartets 40 years later: from 5′-GMP to molecular biology and supramolecular chemistry, *Angew. Chem., Int. Ed. 43*, 668−698.
13. Hardin, C. C., Perry, A. G., and White, K. (2001) Thermodynamic and kinetic characterization of the dissociation and assembly of quadruplex nucleic acids, *Biopolymers 56*, 147−194.
14. Keniry, M. A. (2001) Quadruplex structures in nucleic acids, *Biopolymers 56*, 123−146.
15. Simonsson, T. (2001) G-Quadruplex DNA structures: variations on a theme, *Biol. Chem. 382*, 621−628.
16. Shafer, R. H., and Smirnov, I. (2001) Biological aspects of DNA/RNA quadruplexes, *Biopolymers 56*, 209−227.
17. Sen, D., and Gilbert, W. (1992) Guanine quartet structures, *Methods Enzymol. 211*, 191−199.
18. Neidle, S., and Parkinson, G. N. (2003) The structure of telomeric DNA, *Curr. Opin. Struct. Biol. 13*, 275−283.
19. Williamson, J. R. (1994) G-quartet structures in telomeric DNA, *Annu. Rev. Biophys. Biomol. Struct. 23*, 703−730.
20. Moyzis, R. K., Buckingham, J. M., Cram, L. S., Dani, M., Deaven, L. L., Jones, M. D., Meyne, J., Ratliff, R. L., and Wu, J. R. (1988) A highly conserved repetitive DNA sequence, (TTAGGG)n present at the telomeres of human chromosomes, *Proc. Natl. Acad. Sci. U.S.A. 85*, 6622−6626.
21. Balagurumoorthy, P., and Brahmachari, S. K. (1994) Structure and stability of human telomeric sequence, *J. Biol. Chem. 269*, 21858−21869.
22. Ren, J., Qu, X., Trent, J. O., and Chaires, J. B. (2002) Tiny telomere DNA, *Nucleic Acids Res. 30*, 2307−2315.
23. Parkinson, G. N., Lee, M. P. H., and Neidle, S. (2002) Crystal structure of parallel quadruplexes from human telomeric DNA, *Nature 417*, 876−880.
24. Wang, Y., and Patel, D. J. (1992) Guanine residues in $d(T_2AG_3)$ and $d(T_2G_4)$ form parallel stranded potassium cation stabilized G-quadruplexes with anti glycosidic torsion angles in solution, *Biochemistry 31*, 8112−8119.
25. Wang, Y., and Patel, D. J. (1993) Solution structure of the human telomeric repeat $d[AG_3(T_2AG_3)_3]$ G-tetraplex, *Structure 1*, 263−282.
26. Phan, A. T., and Patel, D. J. (2003) Two repeat human telomeric d(TAGGGTTAGGGT)sequence forms interconverting parallel and antiparallel G-quadruplexes in solution: distinct topologies, thermodynamic properties, and folding/unfolding kinetics, *J. Am. Chem. Soc. 125*, 15021−15027.
27. Alberti, P., and Mergny, J. L. (2003) DNA duplex-quadruplex exchange as the basis for a nanomolecular machine, *Proc. Natl. Acad. Sci. U.S.A. 100*, 1569−1573.
28. Ying, L., Green, J. J., Li, H., Klenerman, D., and Balasubramanian, S. (2003) Studies on the structure and dynamics of the human telomeric G quadruplex by single-molecule fluorescence resonance energy transfer, *Proc. Natl. Acad. Sci. U.S.A. 100*, 14629−14634.
29. Cantor, C. R., Warshaw, M. M., and Shapiro, H. (1970) Oligonucleotide interaction. 3. Circular dichroism studies of the conformation of deoxyoligonucleotides, *Biopolymers 9*, 1059−1077.
30. Marky, L. A., Blumenfeld, K. S., Kozlowski, S., and Breslauer, K. J. (1983) Salt dependent conformational transitions in the self-complementary deoxydecanucleotide d(CGCGAATTCGCG): evidence for hairpin formation, *Biopolymers 22*, 1247−1257.
31. Mergny, J. L., Phan, A. T., and Lacroix, L. (1998) Following G-quartet formation by UV- spectroscopy, *FEBS Lett. 435*, 74−78.
32. Mergny, J. L., De Cian, A., Gheleb, A., Sacca, B., and Lacroix, L. (2005) Kinetics of tetramolecular quadruplexes, *Nucleic Acids Res. 33*, 81−94.
33. Williamson, J. R., Raghuraman, M. K., and Cech, T. R. (1989) Monovalent cation-induced structure of telomeric DNA: The G-quartet model, *Cell 59*, 871−880.
34. Sundquist, W. I., and Klug, A. (1989) Telomeric DNA dimerizes by formation of guanine tetrads between hairpin loops, *Nature 342*, 825−829.
35. Rendon, S., Bombard, S., Elizondo-Riojas, M.-A., and Chottard, J.-C. (2003) Platinum cross-linking of adenines and guanines on the quadruplex structures of the $AG_3(T_2AG_3)_3$ and $(T_2AG_3)_4$ human telomere sequences in Na^+ and K^+ solutions, *Nucleic Acids Res. 31*, 1605−1613.
36. Qi, J., and Shafer, R. H. (2005) Covalent ligation studies on the human telomere quadruplex, *Nucleic Acids Res. 33*, 3185−3192.
37. Zhang, X. Y., Cao, E. H., Zhang, Y., Zhou, C., and Bai, C. (2003) K^+ and Na^+ induced self assembly of telomeric oligonucleotide d(TTAGGG)n, *J. Biomol. Struct. Dyn. 20*, 693−701.
38. Han, H., Cliff, C. L., and Hurley, L. H. (1999) Accelerated assembly of G-quadruplex structures by a small molecule, *Biochemistry 38*, 6981−6986.
39. He, Y., Neumann, R. D., and Panyutin, I. G. (2004) Intramolecular quadruplex conformation of human telomeric DNA assessed with ^{125}I−radioprobing, *Nucleic Acids Res. 32*, 5359−5367.
40. Risitano, A., and Fox, K. R. (2005) Inosine substitutions demonstrate that intramolecular DNA quadruplexes adopt different conformations in the presence of sodium and potassium, *Bioorg. Med. Chem. Lett. 15*, 2047−2050.
41. Lu, M., Guo, Q., and Kallenbach, N. R. (1992) Structure and stability of sodium and potassium complexes of dT_4G_4 and dT_4G_4T, *Biochemistry 31*, 2455−2459.
42. Hardin, C. C., Henderson, E., Watson, T., and Prosser, J. K. (1991) Monovalent cation induced structural transitions in telomeric DNAs: G-DNA folding intermediates, *Biochemistry 30*, 4460−4472.
43. Dapic, V., Abdomerovic, V., Marrington, R., Peberdy, J., Rodger, A., Trent, J. O., and Bates, P. J. (2003) Biophysical and biological properties of quadruplex oligodeoxyribonucleotides, *Nucleic Acids Res. 31*, 2097−2107.
44. Hazel, P., Julian, H., Balasubramaniam, S., and Neidle, S. (2004) Loop-length-dependent folding of G-quadruplexes, *J. Am. Chem. Soc. 126*, 16405−16415.
45. Berova, N., Nakanishi, K., Woody, R. W. (2000) *Circular Dichroism; Principles and Applications*, Wiley-VCH, NewYork.
46. Rujan, L. N., Meleney, J. C., and Bolton, P. H. (2005) Vertebrate telomere repeat DNAs favor external loop propeller quadruplex structures in the presence of high concentrations of potassium, *Nucleic Acids Res. 33*, 2022−2031.
47. Soto, A. M., Loo, J., and Marky, L. A. (2002) Energetic contributions for the formation of TAT/TAT, TAT/$CGC^{(+)}$, and $CGC^{(+)}$/$CGC^{(+)}$ base triplet stacks, *J. Am. Chem. Soc. 124*, 14355−1363.
48. Ghosh, Y. K., Liu, D., and Balasubramanian, S. (2004) Formation of an interlocked quadruplex dimer by d(GGGT), *J. Am. Chem. Soc. 126*, 11009−11016.
49. Sen, D., and Gilbert, D. (1990) A sodium-potassium switch in the formation of four-stranded G4- DNA, *Nature 344*, 410−414.
50. Strahan, G. D., Keniry, M. A., and Shafer, R. H. (1998) NMR structure refinement and dynamics of the K+-$[d(G_3T_4G_3)]_2$ quadruplex via particle mesh Ewald molecular dynamics simulations, *Biophys. J. 75*, 968−981.
51. Miyoshi, D., Nakao, A., and Sugimoto, N. (2003) Structural transition from antiparallel to parallel G-quadruplex of $d(G_4T_4G_4)$ induced by Ca^{2+}, *Nucleic Acids Res. 31*, 1156−1163.
52. Schultze, P., Hud, N. V., Smith, F. W., and Feigon, J. (1999) The effect of sodium, potassium and ammonium ions on the conformation of the dimeric quadruplex formed by the *Oxytricha nova* telomere repeat oligonucleotide $d(G_4T_4G_4)$, *Nucleic Acids Res. 27*, 3018−3028.
53. Patel, P. K., and Hosur, R. V. (1999) NMR observation of T-tetrads in a parallel stranded DNA quadruplex formed by *Saccharomyces cerevisiae* telomere repeats, *Nucleic Acids Res. 27*, 2457−2464.

54. Xu, Y., Noguchi, Y., and Sugiyama, H. (2006) The new models of the human telomere d[AGGG(TTAGGG)3] in K^+ solution, *Bioorg. Med. Chem. 4*, 5584−5591.
55. Vorlickova M., Chladkova J., Kejnovska I., Fialova M., Kypr J. (2005) Guanine tetraplex topology of human telomere DNA is governed by the number of (TTAGGG) repeats, *Nucleic Acids Res. 33*, 5851−5860.
56. Luu, K. N., Phan, A. T., Kuryavyi, V., Lacroix, L., and Patel, D. J. (2006) Structure of the human telomere in K^+ solution: an intramolecular (3+1) G-quadruplex scaffold, *J. Am. Chem. Soc. 128*, 9963−9970.
57. Ambrus, A., Chen, D., Dai, J., Bialis, T., Jones, R. A., and Yang, D. (2006) Human telomeric sequence forms a hybrid-type intramolecular G-quadruplex structure with mixed parallel/antiparallel strands in potassium solution, *Nucleic Acids Res. 34*, 2723−2735.
58. Lee, Y. J., Okumus, B., Kim, D. S., and Ha, T. (2005) Extreme conformational diversity in human telomeric DNA, *Proc. Natl. Acad. Sci. U.S.A. 102*, 18938−18943.
59. Zahler, A. M., Williamson, J. R., Cech, T. R., and Prescott, D. M. (1991) Inhibition of telomerase by G-quartet DNA structures, *Nature 350*, 718−720.
60. Paeschke, Simonsson, K., T., Postberg, J., and Rhodes, D., and Lipps, H. J. (2005) Telomere end-binding proteins control the formation of G-quadruplex DNA structures *in vivo*, *Nat. Struct. Mol. Biol. 12*, 847−854.
61. Schaffitzel. C., Berger, I., Postberg, J., Hanes, J., Lipps, H. J., and Pluckthun, A. (2001) *In vitro* generated antibodies specific for telomeric guanine-quadruplex DNA react with *Stylonychia lemnae* macronuclei, *Proc. Natl. Acad. Sci. U.S.A. 98*, 8572−8577.
62. Chang, C.-C., Kuo, I.-C., Ling, I.-F., Chen, C.-T., Chen, H.-C., Lou, P.-J., Lin, J.-J., and Chang, T.-C. (2004) Detection of quadruplex DNA structures in human telomeres by a fluorescent carbazole derivative, *Anal. Chem. 76*, 4490−4494.
63. Zaug, A. J., Podell, E. R., and Cech, T. R (2005) Human POT1 disrupts telomeric G-quadruplexes allowing telomerase extension *in vitro*, *Proc. Natl. Acad. Sci. U.S.A. 102*, 10864−10869.
64. Kirk, K. E., Harmon, B. P., Reichardt, I. K., Sedat, J. W., and Blackburn, E. H. (1997) Block in anaphase chromosome separation caused by a telomerase template mutation, *Science 275*, 1478−1481.
65. Maddar, H., Ratzkovsky, N., and krauskopf, A. (2001) Role for telomere cap structure in meiosis, *Mol. Biol. Cell 12*, 3191−3203.
66. Williamson, J. R., Raghuraman, M. K., and Cech, T. R. (1989) Monovalent cation-induced structure of telomeric DNA: the G-quartet model, *Cell 59*, 871−880.
67. Sen, D., and Gilbert, W. (1988) Formation of parallel four-stranded complexes by guanine-rich motifs in DNA and its implications for meiosis, *Nature 334*, 364−366.
68. Baumann, P. (2005) Taking control of G-quadruplexes, *Nat. Struct. Mol. Biol. 12*, 832−833.
69. Miura, T., Benevides, J. M., and Thomas, G. J. (1995) A phase diagram for sodium and potassium ion control of polymorphism in telomeric DNA, *J. Mol. Biol. 248*, 233−238.
70. Arthanari, H., and Bolton, P. H. (2001) Functional and dysfunctional roles of quadruplex DNA in cells, *Chem. Biol. 8*, 221−230.
71. Simonsson, T. (2003) A substrate for telomerase, *Trends Biochem. Sci. 28*, 632−638.
72. Kaushik, M., Kukreti, R., Grover, D., Brahamachari, S., and Kukreti, S. (2003) Hairpin-duplex equilibrium reflected in the A → B transition in an undecamer quasi-palindrome present in the locus control region of the human β-globin gene cluster, *Nucleic Acids Res. 31*, 6904−6915.
73. Kaushik, M., and Kukreti, S. (2006) Structural polymorphism exhibited by a quasipalindrome present in the locus control region (LCR) of the human β-globin gene cluster, *Nucleic Acids Res. 34*, 3511−3522.
74. Li, J., Correia, J. J., Wang, L., Trent, J. O., and Chaires, J. B. (2005) Not so crystal clear: the structure of the human telomere G-quadruplex in solution differs from that present in a crystal, *Nucleic Acids Res. 33*, 4649−59.
75. Miyoshi, D., Nakao, A., Toda, T., and Sugimoto, N. (2001) Effect of divalent cations on antiparallel G-quartet structure of d($G_4T_4G_4$) *FEBS Lett. 496*, 128−133.

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