Intramolecular quadruplex conformation of human telomeric DNA assessed with $^{125}$I-radioprobing

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ABSTRACT

A repeated non-coding DNA sequence d(TTAGGG)$_n$ is present in the telomeric ends of all human chromosomes. These repeats can adopt multiple inter and intramolecular non-B-DNA conformations that may play an important role in biological processes. Two intramolecular structures of the telomeric oligonucleotide dAGGG(TTAGGG)$_3$, antiparallel and parallel, have been solved by NMR and X-ray crystallography. In both structures, the telomeric sequence adopts an intramolecular quadruplex structure that is stabilized by G-4 quartets, but the ways in which the sequence folds into the quadruplex are different. The folds of the human telomeric DNA were described as an antiparallel basket-type and a parallel propeller-type. We applied $^{125}$I-radioprobing to determine the conformation of the telomeric quadruplex in solution, in the presence of either Na$^+$ or K$^+$ ions. The probability of DNA breaks caused by decay of $^{125}$I is inversely related to the distance between the radionuclide and the sugar unit of the DNA backbone; hence, the conformation of the DNA backbone can be deduced from the distribution of breaks. The probability of breaks measured in the presence of Na$^+$ and K$^+$ were compared with the distances in basket-type and propeller-type quadruplexes obtained from the NMR and crystal structures. Our radioprobing data demonstrate that the antiparallel conformation was present in solution in the presence of both K$^+$ and Na$^+$. The preferable conformation in the Na$^+$-containing solution was the basket-type antiparallel quadruplex whereas the presence of K$^+$ favored the chair-type antiparallel quadruplex. Thus, we believe that the two antiparallel and the parallel conformations may coexist in solution, and that their relative proportion is determined by the type and concentration of ions.

INTRODUCTION

The ends of chromosomes commonly contain repeats of guanine-rich sequences that may play an important role in biological processes, including chromosome organization and maintenance, as well as in cell aging and death [for recent reviews see (1–3)]. The oligonucleotides of that sequence can form quadruplex structures stabilized with G-4 quartets under physiological conditions (4–9). The quadruplexes formed by G-rich oligonucleotides have been extensively studied (reviewed in 10). There are three types of intramolecular G-quadruplex conformations, antiparallel basket-type and chair-type, and parallel propeller-type (Figure 1). In addition, telomeric oligonucleotides can associate with each other to form various intermolecular structures (4,10–13). Besides the possible biological importance, the G-quadruplex is being explored as a potential target for cancer therapy (13–18). Therefore, it is important to understand in detail, the G-quadruplex structure of human telomeric oligonucleotides.

There is a repeated non-coding DNA sequence d(TTAGGG)$_n$ at the ends of all human chromosomes (19). Two molecular structures are available for intramolecular quadruplexes formed by dAGGG(TTAGGG)$_3$ (11,20–21). The first structure is based on NMR data obtained in the presence of Na$^+$ ions. In this structure, the G-quadruplex adopts the antiparallel basket-type conformation (20). The second structure was obtained by X-ray diffraction on a crystal of dAGGG(TTAGGG)$_3$, obtained in the presence of K$^+$ ions. The oligonucleotide folded into a parallel propeller-type conformation (21) (Figure 1) in the crystal. Interestingly, in 1992, two different structures were obtained for the Oxytricha telomeric sequence (G$_4$T$_4$G$_4$)$_n$ by NMR (22) and X-ray crystallography (23). The X-ray structure that was obtained in the presence of K$^+$ folded in the Greek-key manner (antiparallel chair-type), while the NMR structure folded as the Indian-key ornament (antiparallel basket-type) in the presence of Na$^+$ (24).

Numerous studies that employed biochemical and physical methods ranging from chemical and radiochemical probing and crosslinking to UV spectroscopy and atomic force microscopy suggested a structural polymorphism of the telomeric oligonucleotides in solution (10,25–31). In a recent study using platinum cross-linking, only antiparallel conformations of telomeric oligonucleotides was detected in both Na$^+$- and K$^+$-containing solutions (27). The authors of another recently published paper used single-molecule fluorescence energy transfer to detect two distinct conformations that can coexist and interconvert under near-physiological conditions, which they interpreted as antiparallel and parallel structures (25). Although highly informative, these methods...
cannot distinguish fine structural details of different conformations of the quadruplex, for example, chair- and basket-type of the antiparallel conformation. Therefore, the question of the type of structure that the human telomeric oligonucleotides adopt in solution, as a function of ionic conditions, remains open.

In this paper, we applied $^{125}$I-radioprobing to determine the conformation in solution of the human telomeric quadruplex dAGGG(TTAGGG)$_3$, the same sequence that was used in the NMR and crystallographic analyses. The probability of DNA breaks caused by decay of $^{125}$I is inversely related to the distance between the radionuclide and the sugar unit of the DNA backbone (32). Therefore, with radioprobing, as with NMR, it is possible to obtain information on the interatomic distances; and, in principle, to reconstruct the 3D-structure of DNA. Radioprobing was first tested on DNA triplexes and the DNA–cyclic AMP receptor protein complex, and then it was successfully applied to RNA polymerase transcription elongation complexes, to DNA–RecA protein complexes, and to DNA–RNA heteroduplexes (32–37). We have shown that radioprobing is able to detect changes in internucleotide distances as small as 3 Å, in the distance range from 10 to 30 Å (32).

Oligonucleotides that contained four human telomeric repeats were labeled with $^{125}$I at a single internal position. They were allowed to fold into quadruplexes under different ionic conditions and then frozen for decay accumulation. The probabilities of breaks at individual nucleotides were determined by sequencing gel electrophoresis. Analysis of the quadruplex fold was based on the comparison of the probability of breaks with the distance from $^{125}$I to the corresponding nucleotides derived from the available NMR and X-ray quadruplex structures (20–21).

**MATERIALS AND METHODS**

**Oligodeoxyribonucleotides**

All oligodeoxyribonucleotides (ODNs, Table 1) were synthesized on an ABI394 DNA synthesizer (PE Applied Biosystems, Foster City, CA), and purified by gel and
high-performance liquid chromatography (HPLC) (Model 1050, Hewlett Packard, Palo Alto, CA) as described in detail in (38). The concentration of single-stranded ODN was measured at 260 nm on a HP 8452A Diode Array Spectrophotometer, and was calculated with the extinction coefficient calculator software (http://paris.chem.yale.edu/extinct.html).

The modified base G (7-deaza-8-aza-G(pyrazolo[3,4-d]pyrimidine) (ppG), phosphoramidite was purchased from Glen Research (Sterling, VA); ppG-containing ODNs were synthesized following standard procedures. The template ODNs, VII and X, were synthesized with biotin groups so that they can be removed with magnetic Streptavidin Dynabeads (Dynal ASA, Oslo, Norway) as described (39).

Labeling and purification of oligodeoxyribonucleotides

The telomeric ODNs were labeled with $^{125}$I using $[^{125}$I]-IdCTP and Klenow fragment of DNA polymerase I by primer extension reaction (33). The detailed protocol for $^{125}$I labeling was as follows: 2 μl of 10× Klenow fragment of DNA polymerase I buffer [500 mM Tris–HCl (pH 8.0), 50 mM MgCl2 and 10 mM DTT], 2 μl of 20 μM duplexes (primers V, VI and IX and templates VII and X (Table 1) were annealed before use in equimolar amounts) and 16 μl of H2O were added to freshly-dried 120 Ci $[^{125}$I]-IdC (2 min), and 2 μl of 100 mM dNTP, 1 μl of 10 U/μl Klenow fragment of DNA polymerase I (Fermentas, Hanover, MD) was added. After 15 min at RT, 1 μl of 10 U/μl Klenow fragment of DNA polymerase I buffer [500 mM Tris–HCl (pH 8.0), 50 mM MgCl2 and 10 mM DTT] was added, and after 15 min incubation, the reactions were stopped with 1.5 μl of 0.5 M EDTA. The reaction mixtures were purified by MicroSpin G-25 columns (Amersham Pharmacia Biotech, Piscataway, NJ) to remove free $[^{125}$I]-IdC. The labeled ODNs were separated and further purified from the template using Streptavidin Dynabeads (Dynal) (39) followed by purification with denaturing PAGE. The ODNs were $^{32}$P 5′ end labeled using T4 Polynucleotide Kinase (Fermentas) following the standard protocol.

Gel shift assay

Native gel was prepared with 1× TBE buffer (89 mM Tris, 89 mM boric acid and 20 mM EDTA, pH 8.3), 1× TBE buffer containing 25 mM K2B4O7, or 1× TBE buffer containing 25 mM Na2B4O7 when indicated. The native gels were run at room temperature or at 4°C using a temperature-controlled ThermoFlow Cell (Novex, San Diego, CA).

DMS and DEPC probing

The ODN samples (20 μL, ~1 pmol) labeled with $^{32}$P at the 5′ end were preincubated in 100 mM Na+ or K+ solution for 30 min. Then, 0.5 μl of dimethyl sulfate (DMS) or 0.5 μl of diethyl pyrocarbonate (DEPC) were added and allowed to react for 1 min or 10 min respectively, at room temperature. Then 3 μl of piperidine was added to each sample. After samples were heated at 90°C for 30 min, they were lyophilized to dryness. The pellets were resuspended in 40 μl of distilled water and lyophilized again; this operation was repeated three times. Finally, the samples were dissolved in 5 μl of formamide with dyes, heated to 90°C (2 min), and 2 μl of the samples were loaded onto a denaturing gel.

Preparation of G-4 quadruplexes

The telomeric ODNs were prepared at different concentrations (0, 100 and 150 mM) of NaCl or KCl and incubated at room temperature for 1 h. Then the samples were quickly put in liquid nitrogen for 5 min, and kept at −80°C for 3 weeks.

DNA strand break analysis

After 3 weeks, the samples were thawed and the strand breaks were analyzed by 12 or 20% denaturing PAGE. The DNA strand breaks were quantified using a BAS-2500 Bioimager (FUJI Medical Systems USA, Stamford, CT). To measure the intensity of the individual bands, the intensity profile of each lane was generated from the digitized gel image using Image Gauge software (FUJI Medical Systems USA). The profile was deconvoluted to a series of the Lorentz-type peaks corresponding to individual bands as described in detail in (39). The probability of breaks was calculated from the areas of the individual peaks using a recursive formula and assuming that probability of breaks at $[^{125}$I]-IdC equals 1 as described in detail in (40).
The interatomic distances were calculated from the coordinates from Protein Data Base files 143D (20) and 1KF1 (21). The Coordinates of B-DNA duplex for the human telomeric sequence was generated by V. Zhurkin (NCI).

RESULTS

Gel-shift assay of quadruplex formation

The ODNs containing human telomeric repeats have been shown to adopt multiple intramolecular as well as intermolecular conformations (10–13). Therefore, it is important that the interpretation of radioprobing data proves that the ODNs under investigation adopt intramolecular quadruplex conformation. To address this issue, we applied the band-shift assay in native PAGE and chemical probing with DMS and DEPC, methods that were successfully utilized previously (29,41). As a negative control, we used an oligonucleotide (II) that contains ppG bases instead of G in six positions (Table 1). Unlike G, ppG bases have carbon in the 7th position and, therefore, II cannot participate in the formation of G-tetrads (42).

It was reported that the mobility of a linear ODN in gel was slower than that of an intramolecular G-quadruplex, and faster than that of corresponding intermolecular structures (13). The samples of 32P-labeled ODNs I, II and IV were preincubated in 200 mM KCl or 200 mM NaCl solutions (25 mM K2B4O7 or Na2B4O7). Then the native PAGE was run at room temperature in 1X TBE buffer with or without 25 mM K2B4O7 or Na2B4O7, to determine the formation of quadruplexes. In a 1X TBE gel without addition of extra salt (Figure 2A), telomeric ODNs I and II (lanes 2 and 3) had the same mobility as the ppG-containing ODN II (lanes 6 and 7) and as the 32 nt band of the marker (lane 9), indicating that I and IV did not form quadruplexes during the gel run in 1X TBE only. The quadruplexes I plus complementary template VII and II plus VII (lanes 5 and 8) and the longer template ODN VII (lane 1), as expected, had lower mobility in the gel.

In contrast, when the native gel was run in the presence of 50 mM K+ (Figure 2B) or Na+ (Figure 2C) there was a difference in mobility between I (lanes 3) and II (lane 6); telomeric ODNs I and IV migrated faster than the non-quadruplex forming control, II, with the same sequence and faster than the 32 nt band of the marker (lane 9). This indicates that I and IV did not form quadruplexes during the gel run in 1X TBE only. The quadruplexes I plus complementary template VII and II plus VII (lanes 5 and 8) and the longer template ODN VII (lane 1) as expected, had lower mobility in the gel.

Only a fraction of I formed a duplex with complementary template VII after incubation in KCl (30%), or NaCl (70%) (lanes 5, Figure 2B and C). At the same time, 100% of the ppG-containing ODN II formed the duplex (lane 8). This suggests that the quadruplex is as stable as the duplex in Na+ and even more in K+ especially at the low DNA concentration we used. However, when loaded on a 1X TBE gel at RT the quadruplex became unstable and I quickly forms a duplex with the excess of the complementary VII (lane 5, Figure 2A).

The addition of a short 10 nt oligonucleotide III (Table 1) that was complementary to the tail of I and II (lanes 4 and 7) did not have any effect on the mobility of the later. Most likely

Figure 2. Gel-shift assay for G-quadruplex formation. Autoradiographs of native PAGE run at room temperature in 1X TBE (A); in 1X TBE with 25 mM K2B4O7 (B); and in 1X TBE with 25 mM Na2B4O7 (C). Lane 1: complementary template VII-32P-5’; lane 2: telomeric ODN IV-32P-5’; lane 3: telomeric ODN I-32P-5’ in 200 mM K+ (A and B)/Na+ (C); lane 4: I-32P-5’ complementary to tail ODN III in 200 mM K+ (A and B)/Na+ (C); lane 5: duplex of I-32P-5’ + complementary template VII in 200 mM K+ (A and B)/Na+ (C); lane 6: ppG containing ODN II-32P-5’ in 200 mM K+ (A and B)/Na+ (C); lane 7: II-32P-5’ + complementary to tail ODN III in 200 mM K+ (A and B)/Na+ (C); lane 8: duplex of II-32P-5’ + complementary template VII in 200 mM K+ (A and B)/Na+ (C); lane 9: 8–32 nt oligo dT marker; the positions of 32 nt and 20 nt bands in the marker are shown by arrows.

III is too short to form a stable duplex with the tail for the whole duration of PAGE. In the presence of Na+ (Figure 2C), formation of the quadruplex was not complete and a small portion of the samples (lanes 2–4) migrated on the level of II (lanes 6 and 7), whereas in the presence of K+ almost 100% of the telomeric oligonucleotides formed a quadruplex (Figure 2B, lanes 2–4), and non-quadruplex (lanes 6 and 7) forms is somewhat larger in the presence of K+ than in the presence of Na+ (compare Figure 2B and C). This means that I forms a more stable and more compact quadruplex in K+ than in Na+.

Gel-shift experiments with the two-tailed ODN VIII (data not shown) gave similar results to those shown above for I. Taken together, the gel-shift experiments showed that the oligonucleotides that contains the human telomeric DNA sequence formed an intramolecular quadruplex in the presence of either Na+ or K+.

DMS and DEPC probing of quadruplex

Chemical probing was the original method used to prove the existence of the G-quadruplex (4–9). In G-quartets the N7
positions of all Gs are involved in hydrogen bonding and, therefore, are less reactive to DMS that the Gs in a duplex. DEPC attacks the As perpendicular to the plane of the base and, thus, is significantly more reactive with the As in the loops and single-stranded regions (41).

The results of the chemical probing of the telomeric ODN, **IV**, are shown in Figure 3. The oligonucleotide was subjected to DMS (lanes 1–4) or DEPC (lanes 5–8) reaction in 10 mM Tris–HCl buffer (lanes 1 and 5), or after preincubation in 100 mM NaCl (lanes 2 and 6) or 100 mM KCl (lanes 3 and 7). As controls, reactions were also performed on the duplexes of **IV** with the complementary ODN **VII** (lanes 4 and 8). Even though a nearly equal amount of radioactivity was loaded onto the gel for all the samples, the extent of Gs modification (measured by the intensity of the corresponding bands in the gel) was considerably higher in the duplex (lane 4) than in **IV** (lanes 1–3). In the sample preincubated in the presence of K\(^+\) (lane 3) the Gs of the 3-G repeats in the core of the telomeric oligonucleotide were more protected from the DMS attack, than that in the tail (G(-1)). This modification pattern is in agreement with the tetraplex formation within the core of the telomeric oligonucleotide (30).

As expected, significant modification with DEPC was not observed in the duplex (lane 8). In contrast, in **IV** the As located in the tail and between the 3-G repeats of the core were reactive to DEPC. Again, this modification pattern is consistent with the formation of a structure with three loops and a tail as expected for the G-quadruplex. Therefore, from the gel-shift and the chemical probing data, we conclude that the telomeric ODNs predominantly exist in an intramolecular quadruplex conformation at the specified experimental conditions.

**Radioprobning of the intramolecular quadruplex**

To obtain detailed information on the mode of folding for telomeric ODN **I** into the G-quadruplex structure we applied 125I-radioprobning. The ODNs were labeled with 125I and 32P, purified and allowed to fold in the specified conditions. After folding, the samples were quickly frozen in liquid nitrogen and stored for 3 weeks to accumulate the 125I-decay-induced DNA strand breaks. The thawed samples were analyzed for strand breaks in denaturing PAGE at single nucleotide resolution. The results of radioprobning of 5'-32P end labeled **I**, are shown in Figure 4. Control samples labeled with either 32P (lane 1) or 125I (lane 3) only, did not contain any measurable breaks because the molecules in which the decay had occurred were not visible in the autoradiograph. But, in the 32P labeled sample, some background breaks were still visible due to radiolysis by the longer-range beta particles. In the samples labeled with both radioisotopes (lanes 4–6), the decay of 125I resulted in complete disintegration of the C18 residue that warrants a strand break at this position (40). In addition, the decay of 125I produced strand breaks at other nucleotides of **I**, and the probability of these breaks is an inverse function of the distance to the sugars of the nucleotides. Thus, when **I** was stretched in the duplex with the complementary oligonucleotide **VII** (lane 6), there is one major maximum of breaks at the C18 position. The intensity of bands that corresponds to breaks at the nucleotides between the C18 position and the 32P-labeled 5' end [T17-A(-3)] rapidly decreases, reflecting the increase in the distance from the C18 position to these nucleotides in the linear double helix. In contrast, when **I** was allowed to fold by itself in TE buffer or TE buffer plus KCl (lanes 4 and 5), an additional maxima of breaks can be seen at the loop T5-A7, and at the tail region A1-A(-3), which means that these nucleotides became close to the C18 decay site due to folding. At the same time, the intensity of the maximum of breaks at the C18 position is significantly reduced when compared with the duplex (lane 6), especially in the case of folding in the presence of KCl (lane 5). This is due to the fact that only the fragment that corresponds to the first break from the 32P-labeled 5' end is visible in the autoradiograph.

For further analysis, the probabilities of breaks were calculated from the intensities of the bands by applying the recursive formula as described previously (40). The probabilities of

**Figure 3.** Analysis of DMS and DEPC modification of G-quadruplex. Autoradiograph of 12% denaturing PAGE. Lane 1: telomeric ODN IV-32P-5' + DMS; lane 2: IV-32P-5' + 100 mM NaCl + DMS; lane 3: IV-32P-5' + 100 mM KCl + DMS; lane 4: duplex of IV-32P-5' + complementary template VII + 100 mM KCl + DMS; lane 5: IV-32P-5' + DEPC; lane 6: IV-32P-5' + 100 mM NaCl + DEPC; lane 7: IV-32P-5' + 100 mM KCl + DEPC; lane 8: duplex of IV-32P-5' + VII + 100 mM KCl + DEPC.
breaks for the samples in lanes 4 to 6 of Figure 4 are plotted in Figure 5. The values were normalized such that the probability of break at the [125I]I-C18 equals 1. The curves in the Figure 5 show that the probabilities of breaks rapidly decrease from the [125I]I-C18 position in the duplex. Higher probabilities of breaks at the nucleotides from A(-3) to T12 in TE buffer and KCl reflect folding of I into a compact structure. This folding is more pronounced in KCl than in TE buffer. The first maximum of breaks is in the loop T5-A7 that is adjacent to the [125I]I-C18 in both basket- and chair-type anti-parallel structures. The second maximum of breaks is in the tail region. Therefore, folding of the telomeric DNA can be detected by radioprobing and distribution of probability of breaks that is consistent with the presence of the antiparallel conformations in solution.

**Differences in K\(^+\) and Na\(^+\) quadruplex conformations**

We also applied radioprobing to examine differences in folding of the telomeric ODN I in the presence of K\(^+\) and Na\(^+\). The samples were incubated in Tris buffer containing 100 or 150 mM of KCl or NaCl for 1 h at room temperature to allow for quadruplex formation, then, they were quickly frozen in liquid nitrogen to preserve the structure. The breaks in the samples were analyzed with denaturing PAGE (data not shown), and the resulting probabilities of breaks are shown in Figure 6. As shown in Figure 5, the values were normalized such that the break probability at the [125I]I-C18 was 1. The Y-axis of the graph in Figure 6 is in the linear scale and only a part of the nucleotides of I is shown to highlight the fine structure of break distributions.

In Figure 6, the break distribution in the ppG that contains ODN II is shown as a control. The probability of the breaks at
the nucleotides of II decreases along the DNA chain almost as fast as in the duplex, proving that II cannot fold into a compact structure. Therefore, the N7 positions of all Gs are essential for the formation of the compact structure. This provides additional evidence that the folding of the telomeric oligonucleotide occurred via G-quartet formation.

In contrast, when folded in the presence of both KCl and NaCl, I shows an increased probability of breaks with a maximum in the region of the loop T5–A7. However, the distributions of probabilities of breaks in the presence of K⁺ and Na⁺ are different. In the presence of 100 mM K⁺ the probability of breaks reaches a maximum of 0.053 at A7 followed by a sharp decrease, from 0.051 at T6 to 0.027 at G3. In the presence of 100 mM Na⁺ the maximum probability of breaks shifts to T5 (0.049) followed by a sharp decrease from G4 (0.044) all the way to A(−5) (0.004). In addition, in the presence of K⁺ solution there is a maximum of breaks probability in the tail region, A1–A(−5), and this maximum became more pronounced at a higher concentration of KCl. The addition of a short oligonucleotide (III, Table 1) complementary to the tail eliminates this maximum (data not shown).

Therefore, radioprobing reveals different conformations of telomeric DNA in the presence of KCl and NaCl, respectively. In both K⁺- and Na⁺-conformations the T5–A7 loop is located close to the 125I-containing loop T17–A19. This is consistent with an antiparallel fold. The difference between the two possible antiparallel quadruplex conformations, is that in the basket-type, the T5–A7 and T17–A19 loops run parallel to each other while in the chair-type they are antiparallel (Figure 1). This could explain the observed difference in the distributions of breaks.

Radioprobing of a two-tailed oligonucleotide
To test the hypothesis that the chair-type structure is favored in the presence of K⁺ whereas the basket-type structure is favored in the presence of Na⁺, we synthesized another telomeric sequence-containing oligonucleotide, VIII. Unlike I, VIII has two tails, i.e. two 9 nt complementary sequences flanking the same 4 telomeric repeats core unit. The [125I]-IdC was placed in position +1 of the 3' tail (Table 1, Figure 1). The rationale is that in the chair-type structure the two tails are positioned next to each other and could easily form a duplex, while in the basket-type structure the T11–A13 loop is located between the tails and would interfere with duplex formation in the tail (Figure 1B).

Folding of the two-tailed ODN VIII into an intramolecular G-quadruplex in the presence of K⁺ and Na⁺ was confirmed by the gel-shift assay and DMS chemical probing (data not shown). The results of radioprobing experiments with VIII are shown in Figure 7. When VIII was allowed to fold in the presence of K⁺ there is a strong maximum of breaks probability at the G1–A(-1) positions of the 5' tail (G1 is complementary to the [125I]-IdC at the position +1 in the 3' tail). This maximum is considerably less pronounced when folding occurred in the presence of Na⁺. This indicates that the tails were positioned closer when folding occurred in the presence of K⁺ than in the presence of Na⁺. This is consistent with the chair-type conformation being predominant in KCl while the basket-type conformation is predominant in NaCl (Figure 1B).

DISCUSSION
The majority of DNA breaks resulting from the decay of 125I in the distance range from 10 to 30 Å are produced by low energy electrons, so-called Auger electrons. In addition to Auger electrons, the decay of 125I produces DNA breaks by a charge neutralization of the highly positively charged daughter 125mTe atoms, and by generation of diffusible free radicals in water (43). Charge neutralization is predominant at distances <10 Å from the decay site and mostly affects nucleotides adjacent to the 125I atom (44). The contribution of the free radicals becomes significant at distances greater >30 Å and can be significantly reduced by the presence of free radical scavengers in the solution (44). Due to the complex nature of DNA breaks and the multiple mechanisms involved in their formation, there is no simple analytical relationship between the frequency of breaks at a given nucleotide and the distance to its sugar-phosphate. The results of both theoretical modeling and numerous experiments indicate that the probability of breaks is an inversely proportional monotonically decreasing function of the distance at least for the nucleotides in the same strand (35).

Therefore, to compare our results with the structures obtained by NMR and X-ray crystallography, we plotted the inverse of the average distances from the C5Me position of T18 to the atoms of the sugar-phosphate backbone (without any implication as to the actual mechanism of breaks). The plot is shown in Figure 8. The shortest distances from the C5Me of T18 are to the neighboring nucleotides in the T17–A19 loop. In Figure 8 this is reflected by the absolute maximum of the inverse of average distances for both parallel and antiparallel structures. In the case of the NMR structure there is another inverse distance maximum in the region of the T5–A7 loop reflecting the fact that the T17–A19 and T5–A7 loops are close to each other in the antiparallel basket-type quadruplex fold. There is no antiparallel chair-type structure available, but one would expect a similar inverse distance maximum in the T5–A7 loop, based on the similar arrangement of the loops (Figure 1). In contrast, the graph for the X-ray structure has no such maximum, which indicates that the
T17-A19 and T5-A7 loops are located on opposite sides of the parallel propeller-type quadruplex fold. Therefore, the predicted distribution of breaks in the parallel propeller-type structure would not have any other maxima in addition to the one associated with the T17-A19 loop, and thus would not be easily distinguishable from that of the duplex.

This fact is important for interpretation of our radioprobing data. The breaks distribution maximum in the T5-A7 loop clearly indicates the presence of an antiparallel structure in both K\(^+\)- and Na\(^+\)-containing solutions, which is in agreement with earlier observations that used platinum crosslinking (27). However, we cannot exclude the presence of the parallel conformation in both cases. This is supported by a quantitative analysis of the probabilities of breaks. The probability of breaks in the T5-A7 loop of the quadruplex that folded in the presence of Na\(^+\) is somewhat lower than that of the nucleotides of the duplex that are at the same distance from the decay site. For example, in the antiparallel basket-type quadruplex, the distance from the SMe of T18 to A7 according to the NMR structure is 16.05 Å, and the measured probability of breaks for the fold in the presence of Na\(^+\) is 0.045 (Figure 7). In the duplex, nucleotide G14 is at a longer distance (17.35 Å) and has a higher probability of breaks (0.072). One possible explanation for this discrepancy is a mixture of two quadruplex conformations in solution; one antiparallel with a higher probability of breaks in the T5-A7 loop, and the other, parallel, with a very low probability of breaks in this region. Such a co-existence of the two forms was proposed earlier based on single molecule FRET experiments (25).

There is no 3D structure available for the antiparallel chair-type quadruplex conformation. Thus, we could not perform the distance-based analysis of the breaks distribution for this structure. The experimental data showed a subtle but reproducible difference in breaks distribution when the telomeric oligonucleotide folded in the presence of Na\(^+\) and K\(^+\). Experiments with oligonucleotide of the two-tailed ODN VIII labeled with 125I in a tail, demonstrated that the tails are closer in the structure folded in K\(^+\) than in Na\(^+\), which is consistent with the chair-type antiparallel structure, being more preferable in the presence of K\(^+\). Additional evidence of this comes from the analysis of breaks distribution in the T11-A13 loop. In the basket-type structure this loop crosses from corner to corner at the bottom of the G-quadruplex; and, thus, T11, T12 and A13 are approximately the same distance from the 125I atom (Figure 1). In the chair-type structure, the loop is located at the side of the G-quadruplex, placing A13 closer to the 125I atom than T11. The breaks distributions presented in Figure 7 are consistent with the arrangements described above; in the presence of Na\(^+\), probabilities of breaks are almost the same for T11, T12 and A13, whereas in the presence of K\(^+\), the probability of breaks at A13 is almost two times higher than at T11. However, based on the experimental data, we cannot infer if the telomeric oligonucleotide folds exclusively into the chair-type and basket-type structures in the presence of K\(^+\) and Na\(^+\), correspondingly, or if the two structures coexist with one or the other being predominant at the specified conditions.

In summary, our data demonstrate antiparallel form(s) of human telomeric oligonucleotide to be present in both Na\(^+\)- and K\(^+\)-containing solutions. Quantitative analysis of probability of breaks implies that they may coexist with the parallel form of the quadruplex. Radioprobing shows that, in the presence of Na\(^+\), the preferred antiparallel form of the telomeric oligonucleotide is the basket-type conformation, whereas in the presence of K\(^+\) the chair-type conformation is preferred. We believe that the balance between the two antiparallel and the parallel forms is quite sensitive to ionic conditions during folding, while the inter-transitions between them have been shown to be quite slow (25,41). The challenge now is to determine the biological roles, if any, of the observed telomeric structures.

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