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ORIGINAL PAPER

The first derivative of a function of circular dichroism spectra: biophysical study of human telomeric G-quadruplex

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Abstract Depending on conditions and base modifications, telomeric repeats can form many topological structures; parallel, antiparallel and hybrid forms. The influence of salts and some specific ligands on conformational changes has already been established. In this study, we analyze the human telomeric repeats 5′-GGG(TTAGGG)$_3$-3′ because this sequence forms topologically different structures under various conditions which have been well described by many authors. CD results are compared with electrophoretic and UV absorption spectroscopy results obtained under corresponding conditions in the presence of different ratios of sodium and potassium ions and polyethylene glycol (PEG). We confirmed that the most stable G-quadruplexes could only form under crowding conditions with PEG-200 and K^+ ion, but the molecularity is increased. Other monovalent ions without the presence of K^+ are unable to form the parallel quadruplex conformer and no change of stoichiometry is observed, even when PEG-200 is present. The first derivative of a function applied to CD spectra seems to be a powerful tool for spectra evaluation of any G-quadruplex, and could be more unambiguous than a direct analysis of original spectra.

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Abbreviations

CD	Circular dichroism
FRET	Fluorescence resonance energy transfer
PEG	Polyethylene glycol

Introduction

G-rich DNA sequences can form a large number of structurally diverse G-quadruplex structures in the presence of monovalent ions, e.g. potassium and sodium (Pedroso et al. 2007). Much still remains unclear about the conditions and driving forces of quadruplex topology. Repeats of the 5′-TTAGGG sequence spontaneously form intramolecular/intermolecular quadruplex structures in solution, with a variety of folded morphologies. Different conformations of human telomeric oligomers in water solution and in crystals have been reported by a number of authors (Li et al. 2005; Parkinson et al. 2002; Wang and Patel 1993). In addition, one conformation of G-quadruplex can convert to another, for example, parallel to anti-parallel and intramolecular to intermolecular. Quadruplex folding depends on many factors, including the DNA sequence, the presence of ions and the temperature (Miyoshi et al. 2003; Gray et al. 2009).

Recently, four-repeat human telomere sequences have been shown to form two very similar intramolecular (3 + 1) G-quadruplexes in solutions containing K^+ ion (hybrid form 1 and 2) (Phan et al. 2007). Both structures contain the (3 + 1) G-tetrad core with one double-chain-reversal and two edgewise loops, but differ in the order of loop appearance within the G-quadruplex scaffold. In addition, a basket-type G-quadruplex with only two G-tetrad layers

was detected where loops are successively edgewise, diagonal, and edgewise. Despite the presence of only two G-tetrads in the core, this structure is more stable than the three-G-tetrad intramolecular G-quadruplexes previously observed for human telomeric sequences in potassium solution. This novel structure highlights the conformational heterogeneity of human telomeric DNA (Lim et al. 2009).

Studies have also revealed a crystal structure of the human telomere sequence which has a unique symmetrical propeller-type structure with all parallel G-quartets and three double chain reversal loops (Parkinson et al. 2002). This structure does not seem to be the prevalent form in solution (Li et al. 2005).

In this study, we analyze the human telomeric sequence $G_3(T_2AG_3)_n$ in the presence of various monovalent cations with electrophoresis, CD and UV absorption spectroscopy. This oligomeric sequence serves as a model of the arrangement of quadruplexes in telomeres in solution. CD measurement has frequently been applied to study various telomeric and non-telomeric G-quadruplex forming sequences by many researchers at different ionic conditions and cation—deficient conditions, e.g. (Nagatoishi et al. 2007).

Materials and methods

All chemicals and reagents were obtained from commercial sources. Acrylamide:bisacrylamide (19:1) solution and ammonium persulfate were purchased from Bio-Rad, polyethylene glycol—PEG-200 and N,N,N′,N′-tetramethylethylenediamine were purchased from Fisher Slovakia. DNA oligomer d(G3TTAG3TTAG3TTAG3) was obtained from Sigma, Genosys with milimolar extinction coefficient $\varepsilon = 245.4$ ($mM^{-1}\,cm^{-1}$). DNA oligomer was PAGE-purified and dissolved in double-distilled water before use. Single-strand concentrations were precisely determined by measuring the absorbance (257 nm) at 95°C using molar extinction coefficients (Viglasky et al. 2010).

Circular dichroism spectroscopy

CD spectra were recorded on a Jasco J-810 spectropolarimeter (Easton, MD, USA) equipped with a PTC-423L temperature controller using a quartz cell of 1 mm optical path length in a reaction volume of 300 µl and an instrument scanning speed of 100 nm/min, 1 nm pitch and 1 nm bandwidth, with a response time of 2 s, over a wavelength range of 220–320 nm. The scan of the buffer was subtracted from the average scan for each sample. CD spectra were collected in units of molar circular dichroism versus wavelength. Before CD measurement, each DNA sample was dissolved in an appropriate buffer, heated to 95°C and slowly cooled to the initial temperature of the CD/UV measurement. The same concentration of oligomer was used in each CD experiment; absorbances were chosen to give absorption of around ~0.5 at ~260 nm. Circular dichroism was expressed as the difference in the molar absorption of the right-handed and left-handed circularly polarized light, $\Delta\varepsilon$, in units of $M^{-1}\,cm^{-1}$. DNA samples were annealed at 95°C for 5 min, then allowed to cool down for ~2 h to the initial temperature as at the beginning of the experiment. CD data represent three averaged scans taken at a temperature range of (20–95°C) in the presence of potassium and sodium and for less stable structures (−5 to 80°C) in the presence of lithium and cesium salts. All CD spectra are baseline-corrected for signal contributions caused by the buffer. The modified Britton–Robinson buffer was used in all experiments where TRIS is used instead of KOH (NaOH): 25 mM H_3PO_4, 25 mM boric acid, 25 mM acetic acid, and supplemented by appropriate concentrations of CsCl, KCl, NaCl and LiCl; pH was adjusted by TRIS to the final value of 7.0.

Melting curves

The CD melting profiles were collected at 265 and at 293 nm. The thermal stability of different anti-parallel quadruplexes was also measured by recording the UV absorbance and the CD ellipticity at 293 nm as a function of temperature, by a method similar to that previously published (Viglasky et al. 2010; Mergny et al. 1998). The temperature ranged from −5 to 95°C, the heating rate was 0.25°C per minute. The melting temperature (T_m) was defined as the temperature of the mid-transition point. This T_m value was used as an initial parameter of thermodynamic analysis. From the relation $\Delta G^\circ = -RT\ \ln(K) = \Delta H^\circ - T\Delta S^\circ$, it can be deduced that $\ln(K) = -(\Delta H^\circ/R)\cdot(1/T) + (\Delta S^\circ/R)$. Thus, $\ln(K)$ can be expressed as a linear function of $1/T$. The equilibrium constant K can be written as $K = \alpha/(1-\alpha)$ for an intramolecular equilibrium (α is the fraction of folded oligomer). The van't Hoff enthalpy can only be used for two-state equilibrium, where only two species are present, fully folded and fully unfolded states. However, the analysis is biased if intermediate states of conformation mixtures are significantly populated.

Electrophoresis

Native polyacrylamide gel electrophoresis (PAGE) was run in a temperature-controlled vertical electrophoretic apparatus (Z375039-1EA; Sigma–Aldrich, San Francisco, CA). Gel concentration was 16% (19:1 monomer to bis ratio, Applichem, Darmstadt). About two micrograms (1/5 of DNA as was used for CD experiments) was loaded on 14 × 16 × 0.1 cm gels. Electrophoreses were run at 10°C

for 4–10 h (depending on the concentration of ion and PEG-200) at 126 V (~8 V cm^{-1}). DNA oligomers were visualized with silver after the electrophoresis, and the electrophoretic record was photographed with an Olympus Camedia 3,000 camera (Bassam and Gresshoff 2007).

Results and discussion

We recorded the CD spectra, PAGE and thermal melting profiles of a human telomeric sequence $G_3(T_2AG_3)_3$ in modified Britton–Robinson buffer containing different ions: Li^+, Na^+, K^+, Cs^+ and PEG-200. In addition, we evaluated the CD results at a constant ionic strength maintained between 50 and 200 mM, in which various molar ratios between potassium and sodium were used. The formation of G-quadruplexes in presence of lithium and salt-deficient conditions has been reported by many researchers, e.g. (Rachwal et al. 2007; Nagatoishi et al. 2007; Pedroso et al. 2007; Zhou et al. 2008).

CD spectra

The CD spectra of quadruplexes can indicate whether they fold into a parallel or an anti-parallel conformation, because quartet stacking and the polarity of DNA strands are the determining factors of the intensity and the shape of the CD spectrum, in particular the rotation angle between the stacks (Ambrus et al. 2006; Kypr et al. 2009). However, the interpretation of optical properties such as hypochromicity or the shape and the sign of CD bands can be controversial (Vorlíčková et al. 2005). Therefore, to reduce the ambiguity of DNA conformation, the first derivative of each CD spectrum was applied, Fig. 1b–f. Antiparallel quadruplexes exhibit positive CD signals at around 295 nm, with a negative signal at 260 nm, and in addition the (3 + 1) conformer exhibits a shoulder at 265–270 nm (Luu et al. 2006). In contrast, parallel G-quadruplex structures give a positive band at around 265 nm and a negative peak at 240 nm. The parallel conformers are formed in crowding conditions containing potassium and PEG-200 (Renciuk et al. 2009; Kan et al. 2007). Unfolded oligomers do not display these spectral signatures. These spectral features are mainly attributed to specific guanine stacking in various G-quadruplex structures (Kypr et al. 2009; Gray et al. 2008). Figure 1a shows normalized CD spectra of oligonucleotides in modified Britton–Robinson buffer containing no additional monovalent ions, 50 mM of CsCl, KCl, NaCl, LiCl and 50 mM KCl together with 50% (w/v) PEG-200. The profile of the first derivative function shows the DNA conformation precisely. Antiparallel conformers show two positive peaks at ~300 and ~255 nm and two negative peaks at ~280 and ~240 nm in the presence of Li^+ and Na^+, Fig. 1b and c. Parallel conformers show two main peaks; the positive at ~275 nm and the negative at ~255 nm, Fig. 1f. In the first derivative of the so-called hybrid 3 + 1 conformers in the presence of K^+, we can observe features of both parallel and anti-parallel conformers. Here, three positive peaks and two negative peaks are clearly detected at around 300, 275, 255 nm and 285, 245 nm, respectively, Fig. 1d. The positive peak at ~275 nm is probably caused by a parallel strand orientation, highlighted by the red arrow. Similar features are also observed in the presence of Cs^+, but in these conditions the conformer is less stable, see Table 1. A number of other factors can cause controversy over the evaluation of CD spectra; for example, the presence of mixed populations of various conformers and/or the presence of multimeric conformations in solution (Viglasky et al. 2010; Chaires 2010).

Electrophoresis

Electrophoretic separation provides us with information about the molecularity of G-quadruplexes and the presence of multimeric conformers. The electrophoretic results are shown in Fig. 2. Each electrophoresis was performed with different ions, as described in the figure. A random PCR primer with a base length of 20 oligonucleotides and $d(A)_{40}$ were used as standards (Viglasky et al. 2010). These standards served as benchmarks for comparing mobility between the different electrophoretic patterns. The relative mobilities of $G_3(T_2AG_3)_3$ in the absence of any monovalent ions and in the presence of 50–75 mM LiCl are the same, but the CD spectra and their first derivatives do not correspond exactly over the whole observed region, Fig. 1a, b and e. Mobility coalescence is observed in the presence of 50 mM NaCl and 2.5 mM KCl related to molecular standards. Interestingly, correspondence with the CD spectra and their first derivatives are also observed at these conditions, the amplitudes of dichroic spectra are the same, Table 1. However, in the presence of 25–200 mM KCl, in conditions where hybrid conformers have been confirmed, we can observe significantly higher mobility. The mobility of oligomers in the presence of CsCl falls between the mobilities in the presence of 50 mM KCl and 50 mM NaCl, respectively. The CD spectra and their first derivatives in the presence of CsCl and in the absence of monovalent ions are very close; therefore it is highly probable that their conformations are also similar. The slight peak observed in the first derivative in CsCl at ~275 nm is probably caused by the residual amount of parallel interstrand conformers (indicated by the slower smeared bands). The same effect is observed in buffers containing both sodium and potassium ions. The first derivative curve of 50 mM CsCl fits the curve obtained in 25 mM KCl and 175 mM NaCl perfectly (not shown). However, when the molar ratio of K^+/Na^+ is

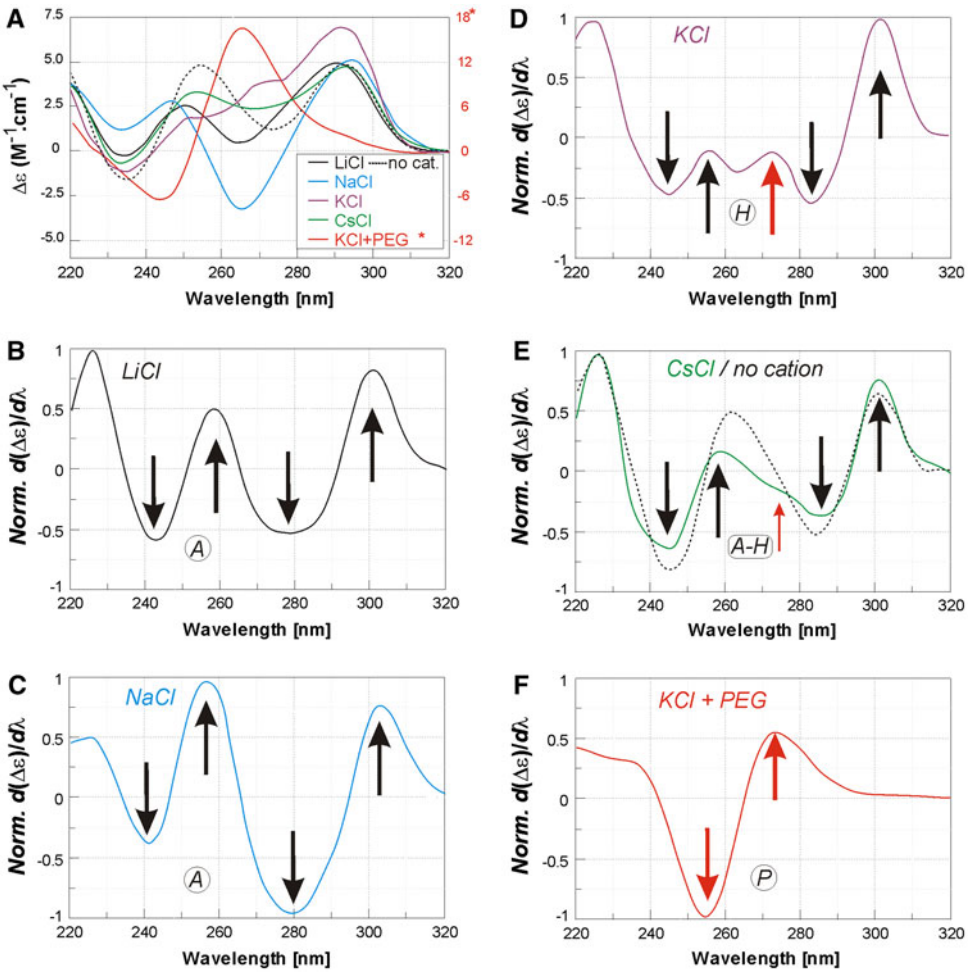

Fig. 1 **a** CD spectra of $G_3(T_2AG_3)_3$ in modified 25 mM Britton–Robinson buffer (pH 7.0) in the presence of 50 mM LiCl (*black line*), NaCl (*blue line*), CsCl (*green line*) and KCl (*magenta line*) and without monovalent ions (*dotted line*). In addition, the *red line* represents a CD spectrum in 50 mM KCl and 50% (w/v) PEG-200; for this spectrum, the right *y*-axes is valid. The first derivative CD spectra corresponding to (**a**) marked by the same color are shown in (**b–f**). Each spectrum corresponds to three averaged scans taken at 2°C and is baseline corrected for signal contribution caused by the buffer

2.5/197.5, the spectra and the first derivatives are identical to the spectra obtained in NaCl in the range of 25–200 mM. However, the melting temperature is significantly higher than is the case in the absence of potassium, Table 1.

The ion competition effect and crowding condition

It is known that potassium ions can stabilize quadruplex structures more efficiently than other ions (Takenaka et al. 2003; Ueyama et al. 2002; Viglasky et al. 2010). This effect could be explained by the fact that the effective radius of the potassium molecule, as opposed to the radii of other ions, both larger and smaller, fits perfectly in the intramolecular space between neighboring G-tetrads; the result is a significant increase in the stability of G-quadruplexes. If quadruplex unfolding and ion dissociation are closely related, then potassium must possess the highest binding affinity. Previous studies using the fluorescence resonance energy transfer method (FRET) have shown that the stability constant (K) of quadruplex-cation complexes significantly depends on G-quadruplex forming sequences; for K^+ against Na^+ of 43,000 ($K^K = 1.3 \times 10^7\ M^{-2}$ vs. $K^{Na} = 3.0 \times 10^2\ M^{-2}$) (Ueyama et al. 2002), but other quadruplex sequences show that the potassium/sodium selectivity ratio is only 150 (Takenaka et al. 2003). FRET measurements have shown that only two cations, Na^+ and K^+, enhance the FRET signal in potassium sensing oligonucleotides (Juskowiak et al. 2006). Interestingly, the above-mentioned authors state that other cations, especially Li^+, gave FRET signals of the same level in the absence of salt, or even below this level (mainly for divalent cations). We have similarly observed that the intensity of CD signal at ~295 nm at 0°C in presence of Li^+, Cs^+ and salt-deficient condition are comparable, Table 1.

If no potassium is present, then the oligomers fold into antiparallel conformers. However, potassium forces the oligomer structure to re-fold into the so-called 3 + 1 hybrid form, where at least three of the four possible parts of DNA strands are oriented in parallel (Ambrus et al. 2006; Gray et al. 2009). Due to the significantly higher association constant of K^+ in comparison with other ions, these other ions can be easily displaced. This means that a small population of K^+ increases the G-quadruplex stability together with the exchange of its topology, Table 1. Experiments in

Table 1 Apparent melting temperature and enthalpy change of G-quadruplex were calculated from UV and CD melting curves which were collected at 293 nm

Britton–Robinson +	Conf.[c]	CD		UV		CD	
		T_m (°C)	ΔH (kcal/mol)	T_m (°C)	ΔH (kcal/mol)	$\Delta\varepsilon_{max}$ (M^{-1} cm^{-1})	F
0	A	23.8	33.6	23.8	33.8	4.9*	0.68
KCl [mM]							
2.5	A	46.1	48.6	46.3	45.7	5.3	0.74
25	H	60.9	55.0	61.4	55.2	6.7	0.93
40	H	63.0	50.0	63.7	50.0	6.7	0.93
50	H	64.7	59.3	65.3	58.8	7.2	1.00
100	H	68.2	57.2	68.6	56.8	7.2	1.00
LiCl [mM]							
50	A	24.8	28.5	26.9	27.4	5.0[a]	0.69
100	A	31.0	26.8	33.5	28.3	5.6[a]	0.77
CsCl [mM]							
50	A-H	26.6	29.5	27.4	27.2	4.8[a]	0.66
	Conf.	T_m (°C)	ΔH (kcal/mol)	T_m (°C)	ΔH (kcal/mol)	$\Delta\varepsilon_{max}$ (M^{-1} cm^{-1})	F
KCl:NaCl [mM][d]							
0:50	A	53.9	44.4	53.6	51.6	5.3	0.75
10:40	A-H	56.8	47.7	56.1	41.6	7.3	1.01
20:30	H	59.0	52.6	59.3	50.1	7.2	1.00
30:20	H	61.0	54.3	61.4	48.0	7.1	0.99
40:10	H	62.4	53.4	61.3	44.4	7.2	1.00
KCl:NaCl [mM][e]							
0:200	A	65.3	46.7	65.8	45.7	5.6	0.78
2.5:197.5	A-H	66.2	46.6	66.9	46.7	5.9	0.82
25:175	H	65.9	53.3	66.5	47.3	6.9	0.96
50:150	H	67.3	49.3	68.1	51.0	7.2	1.00
100:100	H	69.3	54.3	69.1	51.5	7.2	1.00
200:0	H	72.1	44.6	72.2	43.5	7.2	1.00
50 mM XCl + 50%[f] PEG							
LiCl	A	44.3	41.1	40.1	38.7	5.7	0.79
NaCl	A	67.6	45.3	65.2	47.8	6.3	0.86
KCl	P	88.9	59.1	87.4	47.3	16.1[b]	2.23
CsCl	A	45.6	41.7	45.8	48.1	6.6	0.92
KCl+15%PEG	H	65.7	45.3	66.6	48.2	7.5	1.05

F ratio of $\Delta\varepsilon_{obs}/\Delta\varepsilon_{max}$, where $\Delta\varepsilon_{obs}$ is observed dichroic signal and $\Delta\varepsilon_{max}$ is signal observed in modified Britton–Robinson buffer containing 50 mM of KCl

[a] Measurements were performed at −5°C

[b] Molar value of circular dichroism was at 265 nm, ΔH and T_m were obtained from the UV absorption melting curve collected at 263 nm

[c] Conformations are anti-parallel (A), parallel (P), hybrid (H) and mixed population (A-H), where the first derivative function fits the curve between parallel and anti-parallel, as depicted in Fig. 1e at condition with Cs^+

[d] The concentration of potassium and sodium cations is 50 mM

[e] The concentration of potassium and sodium cations is 200 mM

[f] 50 mM of metal cation and 50% (15%) w/v of PEG-200

which the same ionic strength (50 mM and 200 mM) was maintained but the various ratios between different ions altered support this fact. CD derivative spectra where the 3 + 1 hybrid conformer is present in over-abundance show one significant peak at ~300 nm and couple-peaks at ~275 and ~255 nm. PEG-200 mimics the crowding condition occurring in the cellular surroundings (Kan et al. 2007; Miyoshi et al. 2002). PEG-200 causes the increase of either

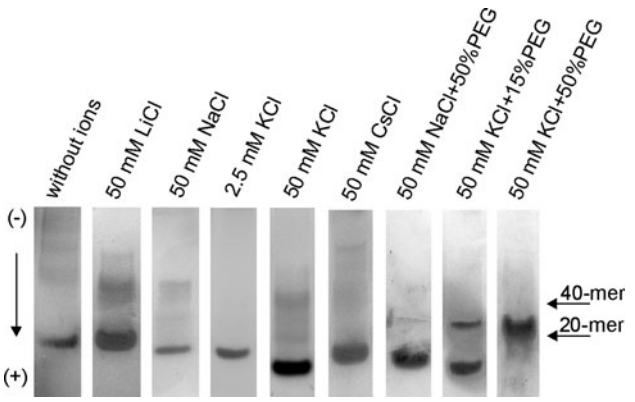

Fig. 2 G-quadruplex resolved by gel electrophoresis and visualized by silver staining. Mobility of the $G_3(T_2AG_3)_3$ in polyacrylamide gels at 10°C in 25 mM Britton–Robinson buffer (pH 7.0) and containing salts ordered as follow: 50 mM LiCl, 50 mM NaCl, 50 mM KCl, 50 mM CsCl, 50 mM NaCl + 50% (w/v) PEG-200, 50 mM KCl + 15% (w/v) PEG-200 and 50 mM KCl + 50% (w/v) PEG-200. As standards, random oligomers 20-mer and 40-mer of poly d(T) have been used. Their positions after the electrophoresis are marked by arrows. The DNA sample prior to use was heated on the same buffer for 5 min at ~98°C and slowly cooled to room temperature within 50 min

3 + 1 hybrid or parallel conformers; the result is a reduction of positive peak at 255 nm towards negative values and a significant increase of peak at 275 nm and a diminishing of peak at 300 nm, Fig. 1f. This effect is not observed for other ions. This means the synergic effect of PEG-200 and potassium causes the change in quadruplex topology. There is no existing detail explanation of the unusual intensity of the CD peak at ~265 nm, but in some reports it is explained by intrinsic properties of the G-tetrads stacking (Mergny et al. 1998; Gray et al. 2008). The dichroic signal at ~265 nm in the presence of 50% (w/v) PEG-200 is approximately twice as high as at ~295 nm without PEG-200. This suggests that potassium and PEG-200 facilitate the formation of conformers that give a higher signal in CD. If this suggestion is true, then we should observe at least one more conformer with electrophoresis. The slowly moving topological invariants are clearly visible, Fig. 2. The formation of both intermolecular multimer and intramolecular monomers is observed in the buffer containing 15% (w/v) PEG-200. At a concentration of 50% (w/v) PEG-200 and KCl, the complete structural conversion to a parallel-stranded dimeric G-quadruplex is induced. However, only intramolecular monomers are observed when Na^+ ions are introduced to the buffer in place of K^+. Although the electrophoresis does provide valuable data, it does not allow us to distinguish intermolecular dimers from dimers consisting of two individual parallel conformers. Nevertheless, the CD measurements show no signal at ~295 nm, suggesting that all parts associated in the formation of stacked G-tetrads strand are aligned in parallel, which would exclude dimers consisting of two hairpin molecules. Therefore, an intermolecular dimer is formed either from two individual parallel conformers or from two interlocked DNA strands, Fig. 3. However, two or more equivalent propeller-like structures should in principle form the same amount of dimers, trimers, etc. This effect was neither observed nor confirmed by electrophoretic separation; no DNA ladder was observed. DNA ladder in presence of potassium may be observed for other G-quadruplex forming sequences, but we did not observe this in the wide range of K^+ concentrations for human telomeric sequence containing four G-runs. Thus, we suggest that interlocked dimers are formed in the presence of K^+ and PEG-200.

Thermal stability

In most cases sigmoidal curves were obtained at ~295 nm with UV absorption and CD spectroscopy for anti-parallel conformers; all results obtained by both measurements are summarized in Table 1. As expected for the dissociation of a G-quartet, the T_m was dependent on the nature of the monovalent ion (Luu et al. 2006). A T_m of 64.7°C was obtained in a Robinson–Britton buffer containing 50 mM KCl, as compared to 53.9°C in a buffer with 50 mM NaCl. Thus, as expected, the effect of ions on the quadruplex stability could be summarized as $K^+ > Na^+ >> Cs^+ \approx Li^+$, regardless of whether PEG-200 was present in solution or not.

Values of ΔH in the table summarize the average values of CD and UV absorption melting data obtained at 265/295 nm and 295 nm, respectively. If the dichroic maximum was observed at 265 (295) nm, then the CD melting curves

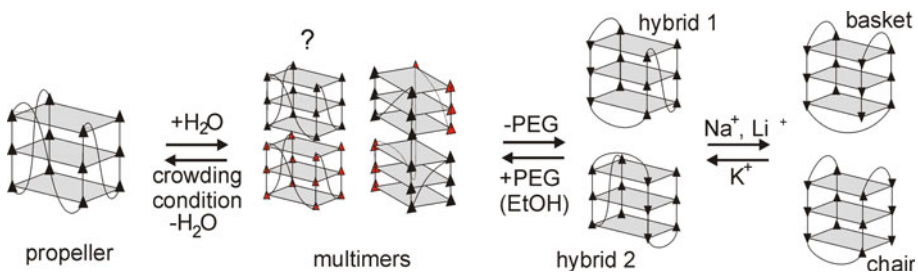

Fig. 3 Schematic drawing of possible G-quadruplex structures induced by monovalent cations and PEG-200

were obtained at 265 (295) nm. Despite this, each melting curve was measured three times; we cannot explain the high values and the unsystematic and wide range of the enthalpy changes. We expected a systematic increase of enthalpy values with increasing levels of K^+, but these values fluctuate. Surprisingly, the minimum value of ΔH was not recorded in the Britton–Robinson buffer without cations, but instead in the presence of Li^+ or Cs^+. It seems that these ions induce a destabilization of the G-quadruplex structure, perhaps due to the water molecule displacement from the cavity of G-quadruplex. In the presence of Na^+, the enthalpy change achieves a value of ~46 kcal/mol and in K^+ a slightly higher value of ~50 kcal/mol. Although the presence of PEG-200 increases the melting temperature; it has no significant effect on enthalpy value in the presence of Na^+ and K^+.

An increase of PEG-200 concentration in presence of 50 mM KCl causes significant change in CD spectra, Fig. 4 and Figure in Supplemental material. Conversion of hybrid conformation to parallel structure causes an increase of the peak at ~265 nm and a decrease of the peak at ~295 nm. Data are collected at 10°C temperature intervals. At 15% (v/w) of PEG-200 there are two comparable peaks detected at 295 and 265 nm. 30–50% PEG induces a significant decrease in CD signal at 295 nm and a signal at 265 nm is approximately twice as high. However, electrophoresis at 15% of PEG-200 shows two clear bands with different electrophoretic mobility. Therefore, we are suggesting that two different conformers occur in solution under this condition. These data agree with the previous analysis, where we use the sequence $(G_4T_2)_3G_4$ instead of $d(G_3T_2A)_3G_3$ (Viglasky et al. 2010). A two-fold CD intensity at 265 nm indicates that G-quadruplex is formed from two individual oligomers folded into an intra-molecular interlocked dimer. However, CD melting curves are biphasic at 263 nm in the presence of 10–30% PEG-200, therefore thermodynamic parameters were calculated only from the CD melting curve at 50% PEG-200.

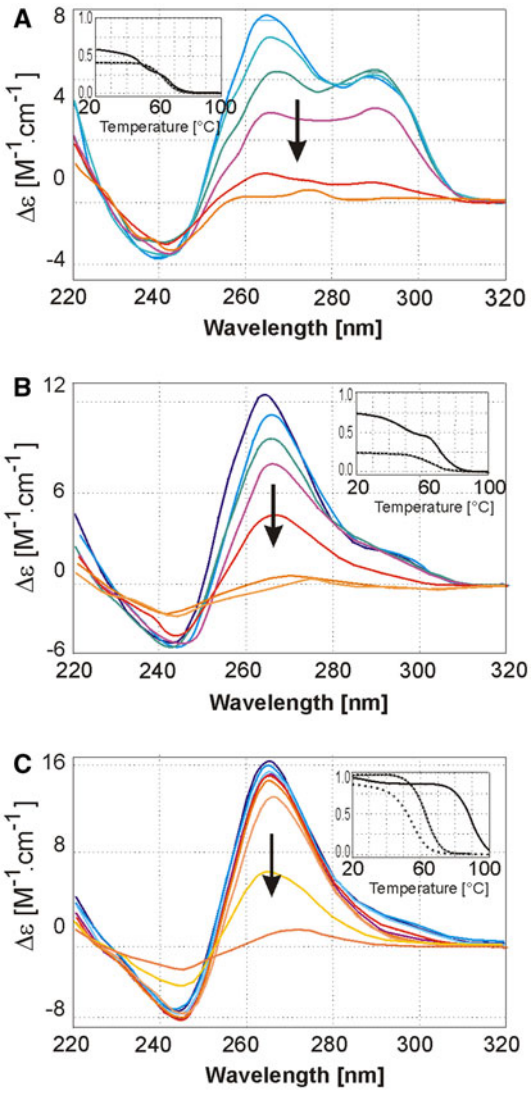

Fig. 4 CD spectra collections of $G_3(T_2AG_3)_3$ in modified 25 mM Britton–Robinson buffer (pH 7.0), 50 mM KCl in the presence of PEG-200: **a** 15% (v/w), **b** 30% (v/w) and **c** 50% (v/w). The corresponding CD melting curves in *insets* of each panel are measured at 293 nm (*dashed lines*) and 265 nm (*solid lines*). *Dashed* and *dotted* lines in the *inset* of **c** show normalized melting curves in presence of 50 mM KCl and NaCl, respectively which were obtained in buffer without PEG-200. CD spectra were collected at different temperature (20, 30, 40, 50, 60, 70, 80 and 90°C); arrow at each panel shows an increase of temperature. The thermodynamic parameters ΔH and T_m of the individual G-quadruplex were determined from the melting curves generated by monitoring the 293 nm by fitting analysis

Aspects of curve fitting analysis

Curve analysis typically assumes a two-state equilibrium between the folded and the unfolded forms; the result is an S-shaped dependence of the melting process. However, the presence of polymorphic quadruplex structures usually leads to a melting profile which deviates from this dependence. Non-sigmoidal shapes of the melting curve have been reviewed by many authors (Chaires 2010; Lane et al. 2008). If DNA quadruplexes unfold in a two-state mechanism, then the isoelliptic (isodichroic) points should be clearly detected, Supplementary figure. However, our spectra collections during the melting curve measurement show that this is not perfectly valid for $G_3(T_2AG_3)_3$ oligomer (Mergny et al. 1998; Viglasky et al. 2010). The mixed population of conformational states in solution confirmed by electrophoresis could explain this fact. A small amount of other conformers and the multi-state mechanism of folding/unfolding can be confirmed by SVD method (single value decomposition) (Antonacci et al. 2007). Herein, the criteria concerning the suitability of a two-state fit is a chi-square parameter.

Overall conclusions

Despite the derivation not bringing quantitatively new information, the first derivative of a function applied to CD spectra of G-quadruplexes seems to be a powerful tool for spectra evaluation, and in some cases other details arise which were not evident from the original CD spectra. The signatures of the first derivative spectrum allow us to distinguish clearly between different conformers.

The effect of monovalent cations and PEG-200 mimicking crowding conditions was investigated. We found that the most stable G-quadruplexes could only form under crowding conditions with PEG-200 and K^+ at concentrations near the physiological concentration. Recent studies have raised significant controversy regarding the exact structure of the quadruplex formed by human telomeric DNA in a physiological relevant environment. Studies on the crystal prepared in K^+ solution revealed a distinct propeller-shaped parallel-stranded conformation. However, many later works failed to confirm such structures in physiological K^+ solution, but rather led to the identification of a different hybrid-type mixed parallel/anti-parallel quadruplex. Other monovalent ions without the presence of K^+ are unable to form the parallel quadruplex conformer even when PEG-200 is present. Here we demonstrate that human telomere DNA can adopt a parallel-stranded conformation in K^+ solution under molecular crowding conditions created by PEG-200. At the concentration of 50% (w/v), PEG induced complete structural conversion to a parallel-stranded G-quadruplex; interestingly, the $G_3(T_2AG_3)_3$ oligomer preferentially formed interlocked dimer (Viglasky et al. 2010).

This observation reveals a physical basis for the formation of stable parallel G-quadruplexes in the genome and supports its presence under in vivo molecular crowding conditions.

We also demonstrate that the quadruplex formed under such conditions has unusual stability and could have a significant negative impact on telomerase processivity. Since the environment inside cells is molecularly crowded, our results obtained under cell mimicking conditions suggest that the parallel-stranded quadruplex may be the more favored structure under physiological conditions.

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