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Conformational Polymorphysm of G-Rich Fragments of DNA Alu-Repeats. II. The Putative Role of G-Quadruplex Structures in Genomic Rearrangements

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Abstract—Three evolutionary conserved (G-rich) sites of Alu repeats (PQS2, PQS3, and PQS4) could form in vitro stable inter- and intramolecular G-quadruplexes (GQs). Structures and topologies of these GQs were elucidated using spectral methods. The study of self-association of G-rich Alu fragments performed using a FRET-based method revealed dimeric GQ formation from two distally located sites $(PQS2)_2$, $(PQS3)_2$ or PQS2–PQS3 within one extended single stranded DNA. Using DOSY NMR, AFM microscopy and differential CD spectroscopy it has been demonstrated that oligomer PQS4 (folded into a parallel intramolecular GQ) forms stacks of quadruplexes stabilized by stacking interactions of external G-tetrads. Comparative analysis of the properties of various GQs suggests involvement of two universal general mechanisms of GQ-dependent genomic rearrangements: (i) formation of dimeric GQs from fragments of different molecules; (ii) formation of GQ-GQ-stacks from pre-folded intramolecular parallel GQs from different strands. Thus, association of G-rich Alu motifs with sensitivity to double-strand breaks and rearrangements may be attributed not to structural features of G-rich Alu fragments, but also to their high abundance.

Keywords: G-quadruplex DNA, Alu-repeats, dynamics of DNA secondary structures

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INTRODUCTION

Intermolecular interactions of G-rich polynucleotide fragments, their involvement in gene regulation, translocation, and recombination are actively discussed in recent publications. Fundamental importance and universality of such processes have been demonstrated in numerous studies. For example, a regulatory function has been demonstrated for DNA-RNA GQ formation [1], GQ-dependent alignment of telomere length and interaction of recombination sites [2]. Recently, a role of GQ-structures in the development of antigenic variation has been described for a wide range of pathogens (gonococci, meningococci, spirochetes) [3, 4]; data on the role of the GQ-sites in the biogenesis of viruses have been summarized [5]. The proposed mechanisms are based on characteristic features of G-rich regions of the polynucleotides; however, patterns of their conformational transformations and formation of intermolecular complexes remain poorly understood. Alu-repeats (Alu) of the human genome are known to play an important role in the processes of translocation, recombination, and development of diseases [6]. In this study we have investigated the conservative Alu PQS (Potential GQ Sites), their structure and properties to assess the possible role of non-canonical structures in the mechanisms of genome rearrangements.

In the first part of this study [7] we have analyzed Alu-sequences of the prooncogenes bcl2 (intron Alu_{bcl2}) and the consensus Alu-repeat (Alu_{cons}) [8, 9]; identified PQS fragments (Fig. 1). It has been also shown that in the human genome they are mainly associated with the active Alu-families. In experiments with cultivated cells gene-regulatory properties of recombinant Alu have been recognized. For the first time it has been confirmed that Alu-amplicons form non-B-structures. In this paper we have considered the ability of PQS Alu to form in vitro intra- and intermolecular GQs, their thermal stability, and topology. We have also analyzed self-association of model oligomers and possibilities of realization of GQ-recombination processes involving PQS Alu.

MATERIALS AND METHODS

Synthesis of oligonucleotides, their purification and analysis were carried as described in [10].

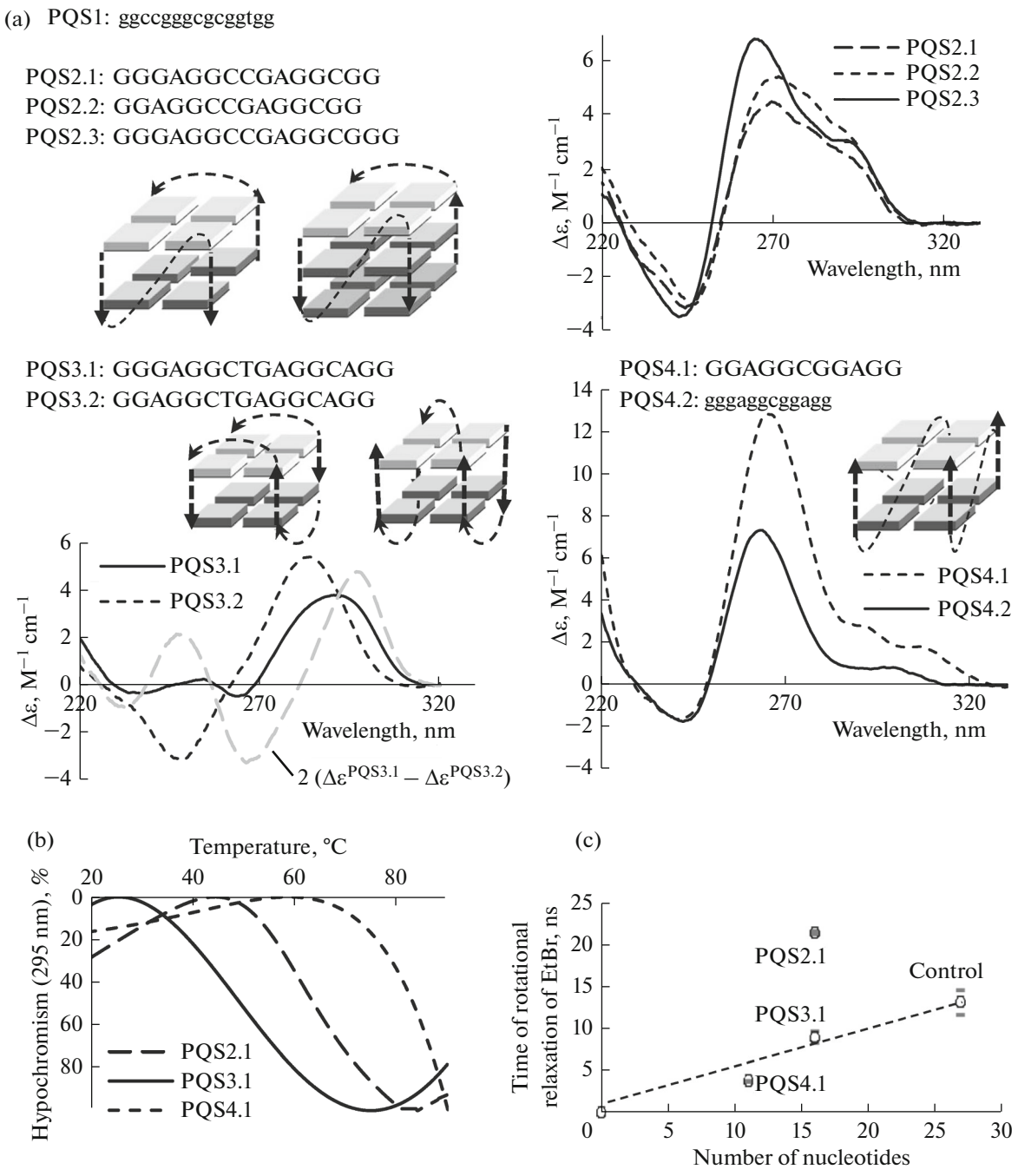

Fig. 1. Analysis of Alu PQS secondary structures by optical methods. (a) CD spectra and a schematic representation of proposed GQ structures. Uppercase letters denote PQS Alu_{cons}, missed in Alu_{bcl2}. PQS1 does not form GQ according to CD and UV melting. Molar ellipticity is given per 1 nucleotide. Conditions: 20 mM Na-phosphate buffer (pH 7.5), 100 mM KCl. The concentration of oligonucleotides: 5 μM, fast annealing immediately before the experiment. (b) UV-melting curves. (c) Analysis of rotational relaxation time of EtBr in complex with oligonucleotides (calculated on the basis of polarization values and EtBr fluorescence lifetime). Points on the graph correspond to the average value of three measurements; the standard deviation does not exceed 10%.

UV and CD Spectroscopy

Circular dichroism (CD) spectra and thermal dissociation profiles (melting curves) of GQs were registered using a Chirascan spectrophotometer (Applied Photophysics, UK). Oligonucleotides were dissolved in 20 mM sodium phosphate buffer (pH 7.5), containing 100 mM KCl. Immediately before the experiment, the samples were heated to 90°C, held for 5 min and cooled on ice (fast annealing for intramolecular folding). In the study of the concentration dependence of

the PQS2.1 melting temperature slow annealing (heating to 90°C and gradual cooling to room temperature) was performed. Rotational relaxation time of EtBr in complexes with the oligonucleotides was determined by a well known procedure [11, 12].

FRET

Solutions of the oligonucleotides (1 μM), carrying a fluorescent label (FAM) at the 5'-end and a quencher (BHQ1) at the 3'-end, in 20 mM Tris-HCl buffer (pH 7.8) containing 100 mM KCl, were subjected to fast annealing as described above. Fluorescence emission spectra and fluorescence melting curves were recorded at 520 nm on a Chirascan spectrophotometer (Applied Photophysics) at the excitation wavelength of 490 nm.

Atomic Force Microscopy (AFM)

Preparation of the oligonucleotide samples consisted in slow annealing of 0.1–0.2 mM oligonucleotide solutions in the buffer containing 10–200 mM KCl, 10 mM Tris-HCl, pH 7.5. An aliquot of the solution was diluted 100–2000 times with buffer (10 mM KCl, 0.5 mM Tris-HCl, pH 7.5) and applied on the surface of the modified graphite. Scanning was performed using an AFM microscope (NT-MDT, Russia) as described in [13].

The sample surface relief was investigated in a semi-contact resonant mode by means of the research complex Integra Prima (NT-MDT, Russia) using NOVA 1.1 management software. Hyperacute high resolution silicon cantilevers with the resonance frequency of 190–325 kHz, tip curvature radius <2 nm (Nanotuning, Russia) were used. The amplitude of free cantilever oscillations in air was 1–10 nm, the automatically maintained amplitude (SetPoint parameter) was set at the level of 60–70% of free oscillations. Signal processing and imaging were performed using NOVA 1.1 software (NT-MDT); heights of visualized objects were evaluated by the image processing with module NOVA Image Analysis 2.0.

NMR Spectroscopy

For NMR spectroscopy studies oligonucleotides were dissolved in 10 mM Tris-HCl-buffer (pH 7.5) 20–40 mM KCl, Tris-HCl. Spectra were obtained using a Bruker Avance III 500 MHz NMR spectrometer, equipped with a Prodigy TCI cryogenic triple-channel probe. For each oligonucleotide sample, one-dimensional proton spectra were recorded with water suppression (WATERGATE with five pairs of binomial gradient pulses) with a relaxation delay of 3 s, the gradient delay of 200 μs and a duration of binomial pulses for water suppression of 125 μs in 256–1024 passages at temperatures from 290 K to 330 K in 10 K steps. For oligonucleotides and standard calibration samples we also registered two-dimensional DOSY diffusion spectra with water suppression using WATERGATE (using pulse sequence with bipolar gradient pulses STEBPG) with a relaxation delay of 3 s and the above given WATERGATE parameters and the diffusion delay (Δ) of 100 ms with changes in the gradient pulse power from 5% to 95% in 16 steps of 64 passages. All the samples were analyzed in ampoules of 5 mm in diameter and using 600 μL of the sample solution, and adding 50 μL of D_2O to stabilize the signal.

Electrophoretic Analysis in PAGE

Self-assembly of oligonucleotides was studied by means of analytical electrophoresis under non-denaturing conditions. After fast annealing, 200 μL of DPQS4.1 oligomer solutions (10 pmol/μL) in buffer (20 mM KCl, 0.1 mM Tris-HCl, pH 7.5) were concentrated in vacuo up to 10 times (2 h at 20°C). Polyacrylamide gel electrophoresis (PAGE) was performed in a standard 10% gel (without urea addition) for 60 min at 4°C and 250 V and stained with SYBR Gold (Invitrogen, USA).

RESULTS AND DISCUSSION

Study of Secondary Structure of Alu PQS

Sequence analysis of Alu_{bcl2} and Alu_{cons} revealed partially overlapping PQS. Secondary structure of each PQS oligonucleotide was characterized by optical methods (Fig. 1).

The PQS1 oligomer existing only in Alu_{cons} does not form GQs. Profiles of its thermal denaturation lack transitions at 295 nm typical for GQ. The PQS1 sequence coincides with the B-box sequence [9, 14] within the Alu element, which is an internal promoter of RNA polymerase III. It should be noted that this result supports the hypothesis that the formation of a non-canonical structures in the polymerase binding locus may complicate DNA interaction with the enzyme and therefore it seems unlikely.

According to the CD-spectroscopy (Fig. 1a) and UV-melting (Fig. 1b) oligonucleotides PQS2–4 form thermostable GQs (m.p. = 50–85°C) under physiological conditions.

The PQS2.1–PQS2.3 CD amplitude ratio at 265 nm and 295 nm suggests a hybrid topology of GQs (provided that in the case of PQS2.3 two G-quartets in the GQ core have the same polarity, while the third one has opposite polarity; Fig. 1a).

Comparison of the rotational relaxation time of the fluorescent intercalator in the complex with Alu PQS-oligonucleotides (in a first approximation this value is proportional to the hydrodynamic volume of the complex) and the control intramolecular structure

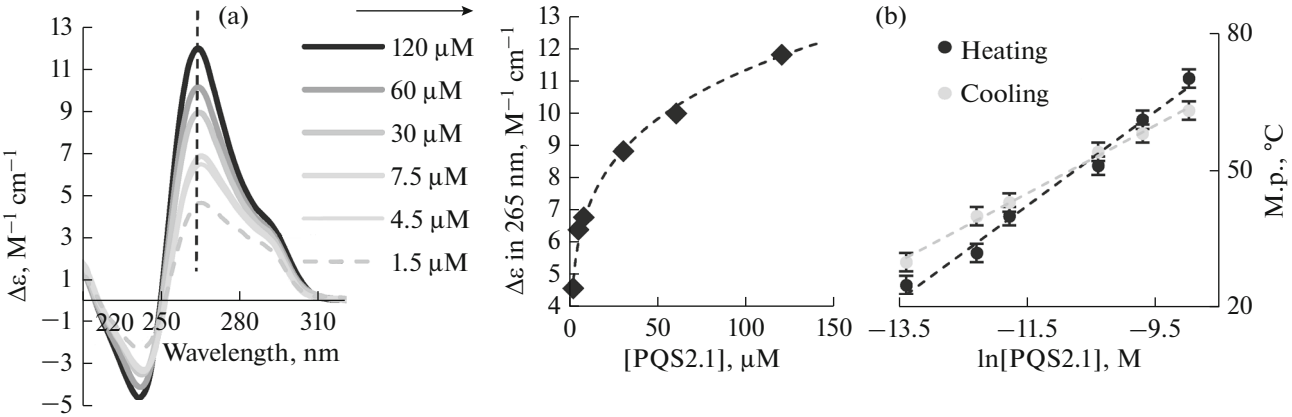

Fig. 2. The study of self-assembly of PQS2.1. by optical methods. (a) The concentration dependence of molar ellipticity of PQS2.1. Conditions: 20 mM Tris-HCl, 100 mM KCl; slow annealing prior to the experiment (after fast annealing, the shape of the curve is the same). (b) Concentration dependence of PQS2.1 melting temperature. The rate of heating/cooling was 0.5°C/min.

(27-membered hairpin) indicates that PQS2.1 represents a bimolecular GQ, while PQS3.1 and PQS4.1 are intramolecular structures (Fig. 1c).

The concentration dependence of the structure (Fig. 2) also suggests biomolecularity of PQS2.1 GQ.

The CD spectrum of PQS3.1 shares similarity with the classical spectrum of the antiparallel GQ (positive maxima around 245 nm and 290 nm and a negative maximum at 265 nm), but the main positive maximum is shifted to shorter wavelength (290 nm instead of expected 295 nm). This effect is associated with the fact that in solution PQS3.1 exists as an equilibrium mixture of conformers: as molten threads or noncanonical hairpins and parallel two-quartet GQ. Figure 1a shows that the observed PQS3.1 spectrum may represent the result of superposition of classical spectrum of the antiparallel GQ and the spectrum of PQS3.2, a PQS3.1 fragment, which does not form GQ (according to the UV-melting).

CD spectra of PQS4.1 and PQS4.2 are typical for parallel GQ (positive maximum at 265 nm, and negative maximum at 245 nm, Fig. 1c). These oligonucleotides are one of the first examples of parallel two-quartet GQs with high thermal stability (m.p. > 80°C) and represent a new promising object for subsequent studies.

Figure 3a shows fragments of one-dimensional proton NMR spectra of the oligonucleotides PQS4.1 and PQS3.1. Eight proton signals of imino groups (11–12 ppm), involved in the formation of hydrogen bonds, correspond to two G-quartets; this is consistent with the structure shown in Fig. 1. In the case of PQS2.1 the exact number of protons involved in the formation of Hoogsteen hydrogen bonds could not be quantified due to signal broadening in the imino region; this may mean polymorphism of the PQS2.1 structure and/or the formation of intermolecular GQs.

Thus, analysis of the G-rich Alu fragments at the level of oligonucleotide models revealed three loci that can participate under physiological conditions in the formation of highly stable GQs of different topologies.

In order to understand the mechanisms of GQ-dependent genomic rearrangements it is necessary to investigate the possibility of formation and the nature of intermolecular GQ-polynucleotide complexes, as well as to assess the ability of PQS_{Alu} to form such complexes.

Self-Association of PQS_{Alu}

Formation of GQ stacks by the PQS4.1 oligomer. Formation of stacks of parallel quadruplexes stabilized by stacking interactions of external G-quartets has been previously detected in several studies [15, 16]. Formation of oligonucleotide G3 (5'GGGTGGGTGGGTGGG) dimers has been described in details [15]. We hypothesized that the compact two-quartet parallel PQS4 are also susceptible to self-assembly into stacks (schematic representation of GQ stacks is shown in Fig. 4b).

Indeed, the increase in oligomer PQS4.1 concentrations was accompanied by a marked increase in molar ellipticity at 265 nm, implying additional stacking of tetrads with the same polarity (Fig. 4a). The concentration dependence of the molar ellipticity was also observed in the case of control parallel quadruplex G3 (gray curve in Fig. 4a). A known anti-parallel GQ TBA (5'GGTTGGTGTGGTTGG) and complementary oligonucleotide LCont (5'CCAACCACACCAACC), not forming GQ, were used as negative controls. As expected molar ellipticity of LCont and TBA, did not demonstrate any concentration dependence and the amplitude of the differential CD spectrum was close to zero. Obviously, the loops protruding above

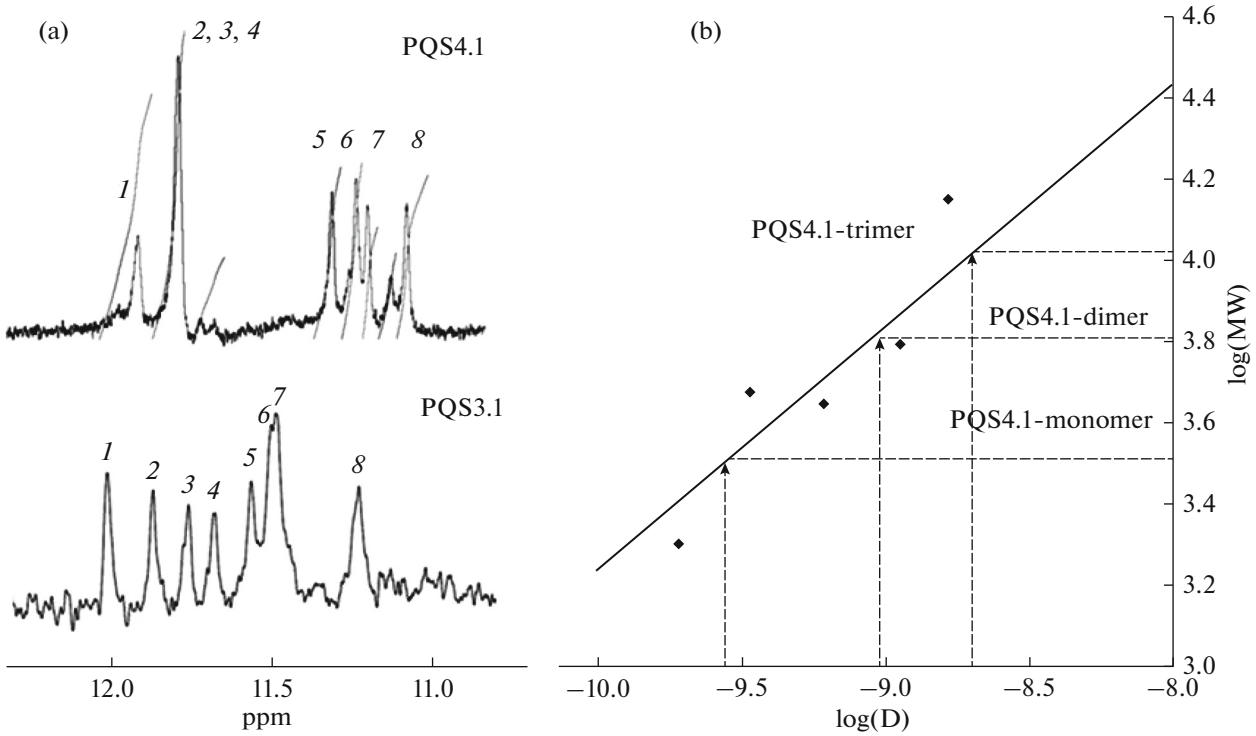

Fig. 3. The study of secondary structure of Alu PQS by NMR spectroscopy. (a) Fragments of one-dimensional proton NMR spectra of PQS4.1 and PQS3.1 oligonucleotides. (b) Analysis of self-association of PQS4.1 by diffusion ordered NMR spectroscopy (DOSY). Calibration straight line of the dependence of the diffusion coefficient (*D*) on the molecular mass (MW) in double logarithmic coordinates has been built on the basis of DOSY NMR data for the polyethylene glycol (2 kDa), lysozyme, and also known monomolecular GQ TBA (GGTTGGTGTGGTTGG) and G3 oligonucleotide (GGGTGGGTGGGTGGG). Experimental values of log*D*, obtained under the same conditions for oligonucleotide PQS4.1, correspond to monomeric (logMW = 3.5), dimeric (logMW = 3.8), and trimeric (logMW = 4.0) structures.

the plane of G-tetrads, prevented inter-quadruplex stacking.

The study of PQS self-assembly by the method of diffusion ordered NMR spectroscopy (DOSY) (Fig. 3b) led us to similar conclusions: in the case of PQS4.1 it was possible to detect formation of dimers and trimers; control parallel GQ (G3) also formed dimers, while antiparallel GQ (TBA) existed in solution only as monomers.

Formation of PQS4.1 stacks was also found during analysis of oligomer preparations by AFM after a slow annealing. The scan, shown in Fig. 4b, demonstrates clearly distinguishable molecules of PQS4.1 GQ and GQ stacks (marked by rectangles).

Thus, self-association of the PQS4.1 oligonucleotide with formation of stable stacks has been demonstrated under physiological conditions by three independent analytical methods.

It is important to note that experimental evidence for inter-quadruplex stacking with formation of dimer (GQ-GQ) and multimeric stacks by the member of two-quartet GQs (PQS4.1) together with literature data suggest the general nature of such interactions for different GQs of parallel topology.

Processes of genomic rearrangements (chromosomal translocations and others) imply complex formation of exchanging strands after the break of the polynucleotide sugar phosphate backbone. During homologous recombination complex formation is determined by Watson-Crick bonds, while in the case of GQ-dependent rearrangements other bonds, presumably intermolecular GQ, are involved. In order to assess potential contribution of stacks of parallel GQs within an extended polynucleotide chain we have examined self-association of a model oligomer carrying two distal PQS4.1 repeats (DPQS4.1, 5'-GGAG-GCGGAGG-TTTTTTTTTTTTTTTTT-GGAG-GCGGAGG). The formation of dimers and multimers can be clearly detected by PAGE electrophoresis under non-denaturing conditions during analysis of composition of DPQS4.1 oligomer solution after annealing in the presence of potassium ions (Fig. 4c). Heating the mixture (5 min, 95°C) resulted in a decrease of mobility of the DPQS4.1 monomer (Fig. 4c, UV detection). The latter is obviously associated with melting of GQ structures in this monomer. It should be noted that a part of the dimeric structures (GQ-GQ) remained stable.

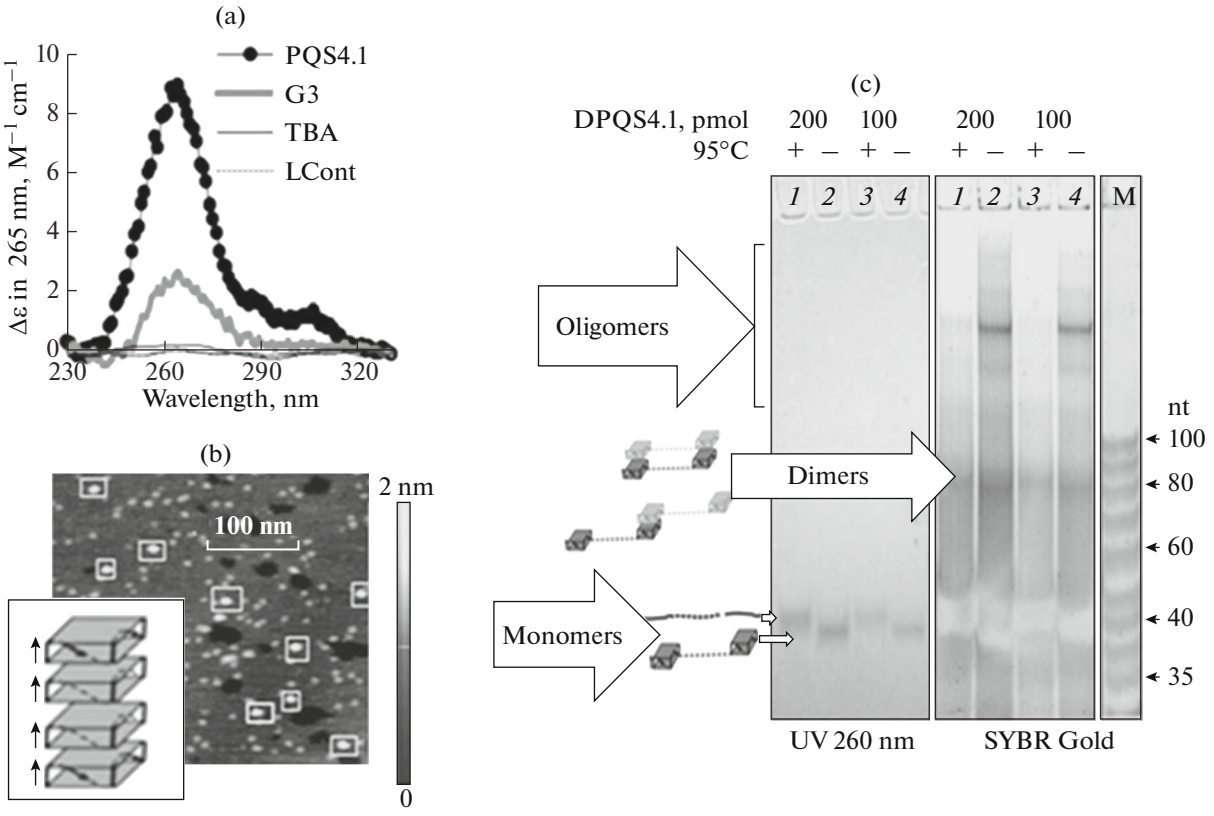

Fig. 4. The study of self-assembly of PQS4.1. (a) Differential CD spectra—the difference of molar ellipticity obtained at PQS4.1 oligonucleotide concentrations of 45 μM and 5 μM. Controls: G3 (5'GGGTGGGTGGGTGGG)—parallel GQ; TBA (5'GGTTGGTGTGGTTGG)—antiparallel GQ; LCont (5'CCAACCACACCAACC)—a single-stranded oligonucleotide which does not form secondary structures. (b) AFM analysis of PQS4.1 oligonucleotide after slow annealing (5 μM oligomer solution, 10 mM Tris-HCl, 200 mM KCl), and a principal scheme of the assembly of GQ stacks. Supramolecular assemblies are marked. (c) Analysis of electrophoretic mobility in 10% PAAG (non-denaturing conditions) of oligomer DPQS4.1 after annealing. Before loading, samples 1 and 3 were heated at 95°C for 5 min. The amount of the oligomer applied onto a well is indicated. Principal schemes of associate structures are given.

Thus, these model studies have shown the possibility of participation of the PQS4 Alu-site in formation of stable GQs and GQ-stacks representing a potential basis for intermolecular GQ-dependent rearrangements of the genome.

Intermolecular GQs Involving Alu PQS2 and PQS3 Sites

PQS2 and PQS3, with a general formula 5'GGGAGGC(C/T)GAGGC(G/A)GG, are fragments of Alu tandem repeats. We have found that core oligomers of these groups form GQs of antiparallel or mixed topology. They cannot be associated in stacks due to lateral/diagonal loops. However, as shown using PQS2.1 as an example, they tend to form tight GQ dimers in solution. In order to investigate whether this property is retained within extended polynucleotide sequences we have prepared a number of oligomers PQS2/3-T_{20}-PQS2/3 (see table) containing a fluorescent label (FAM) and a quencher (BHQ1) at their ends.

The most pronounced fluorescence quenching observed after fast annealing of diluted solutions of oligonucleotides (Fig. 5a) was particularly pronounced in the case of DPQS2 oligomers. This correlates with prevalence of the conformation of bimolecular GQ in PQS2.1 solution (Fig. 1a and Fig. 2). After thermal denaturation of the structures, no FRET effect was observed (Fig. 5b). Melting curves of oligomers (Fig. 5c) also confirm formation of PQS2-PQS2, PQS3-PQS3 and PQS2-PQS3 structures, and CD spectra (Fig. 5d) refer them to mixed type GQs. Assuming that GQ formation involves six terminal nucleotides, the PQS2 sites $(5'\text{-GGGAGG})_2$ and $(\text{GGCGGG-}3')_2$ can be theoretically stacked into three-quartet and five quartet GQs with single-nucleotide bulges. For PQS2_PQS3 and DPQS3, involvement of the GGC<u>A</u>GG-3' site instead of GGC<u>G</u>GG-3' in one and two strands, respectively, should result in decreased thermal stability of GQ

Oligonucleotides used for FRET-analysis

Name	Sequence 5' → 3'
DPQS2	FAM-T<u>GGGAGGCCGAGGCGGG</u>$(T)_{20}$<u>GGGAGGCCGAGGCGGG</u>C-BHQ1
DPQS3	FAM-T*GGGAGGCCGAGGCGGG*$(T)_{20}$*GGGAGGCTGAGGCAGG*A-BHQ1
PQS2_PQS3	FAM-T*GGGAGGCTGAGGCAGG*$(T)_{20}$<u>GGGAGGCTGAGGCAGG</u>A-BHQ1
FRET_Cont1	FAM-TGTCACCGAGACGTTT$(T)_{20}$TAGTCAGTTCGCTAGA-BHQ1
FRET_Cont2	FAM-TCAAAGTCAATCTTGACCACGTCATCG-BHQ1

FRET_Cont1 imitates the distance between the label and the quencher in unstructured form; FRET_Cont2 corresponds to the maximum shortening of the oligomers during the formation of the terminal GQ-structures. PQS2 fragments of the sequences are underlined; PQS3 are shown in italics.

(due to the decrease in the number of tetrads). Indeed, the thermal stability decreased in the following order: DPQS2 > PQS2_PQS3 > DPQS3 (m.p. ~60° to ~35°C, Fig. 5c).

Thus, we have demonstrated the possibility of participation of PQS2 and PQS3 loci in intermolecular interactions of polynucleotides with formation of bimolecular GQs.

CONCLUSIONS

Our systemic study has shown for the first time that three conserved Alu fragments are able to form in vitro

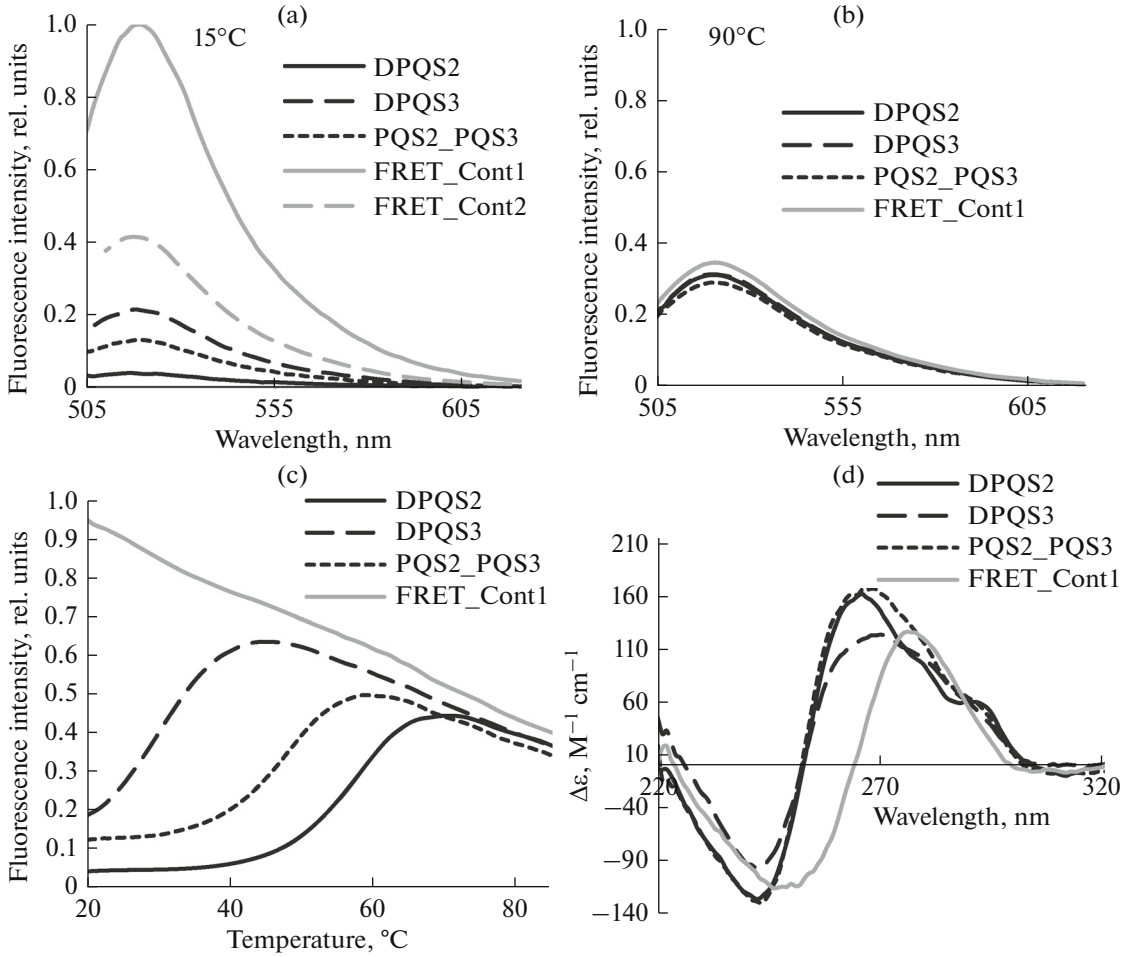

Fig. 5. Formation of intramolecular GQs from distal PQS2/3. (a) Fluorescence spectra of DPQS2 oligomers, DPQS3, PQS2_PQS3, and control oligomers at 15°C. (b) Fluorescence spectra at 90°C. (c) Profiles of thermal denaturation. (d) CD spectra with deduced spectrum of the T_{20} spacer.

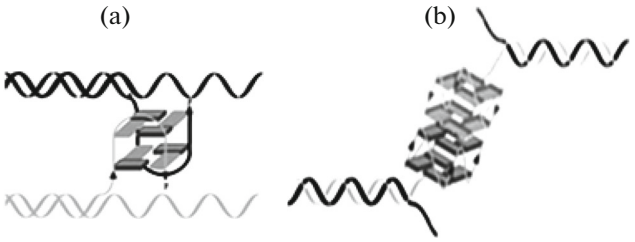

Fig. 6. Principal schemes of intermolecular interactions between fragments of two polynucleotide chains, stabilized by GQ formation. (a) GQ formation involves G-rich sites nicked/broken in both DNA strands or single-stranded loci of DNA and RNA. (b) Intermolecular structure is stabilized by stacking of parallel GQs formed in each strand. The schemes of interactions are common for different structures and two-quartet structures are given as an example.

stable intra- and intermolecular GQs, and secondary structure of G-rich fragments of Alu repeats play a role in gene regulation.

Analysis of the G-rich fragments of Alu repeats at the level of oligonucleotide models revealed three loci that could form highly stable GQ of different topologies under physiological conditions.

The study of self-assembly of Alu PQS, including distally located fragments within the extended single-stranded DNA (DPQS4.1, PQS2-PQS2, PQS3-PQS3 and PQS2-PQS3) with formation of bimolecular GQ (PQS2/3) and stable GQ-stacks (PQS4), allowed to assume their involvement in the GQ-dependent intermolecular rearrangements typical for non-homologous recombination processes and translocation, with possible implementation of two GQ-dependent mechanisms (Fig. 6).

It should be noted that formation of intermolecular GQs (Fig. 6a) is possible for different PQS and G-rich sequences carrying at least two G-blocks. The GQ-GQ stacking (Fig. 6b) can also be considered as a universal mechanism for parallel GQs of different composition.

From this point of view, association of G-rich motifs of Alu repeats with loci sensitive to breaks and rearrangements [12] may be determined by high abundance of G-rich fragments of Alu repeats rather than their structural features.

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REFERENCES

1. Loomis, E.W., Sanz, L.A., Chedin, F., and Hagerman, P.J., *PLoS Genet.*, 2014, vol. 10, e1004294. PGENETICS-D-13-02486 [pii] doi 10.1371/journal.pgen.1004294
2. Murat, P., Zhong, J., Lekieffre, L., Cowieson, N.P., Clancy, J.L., Preiss, T., Balasubramanian, S., Khanna, R., and Tellam, J., *Nat. Chem. Biol.*, 2014, vol. 10, pp. 358–364. nchembio.1479 [pii] doi 10.1038/nchembio.1479
3. Harris, L.M. and Merrick, C.J., *PLoS Pathog.*, 2015, vol. 11, e1004562. PPATHOGENS-D-14-01954 [pii] doi 10.1371/journal.ppat.1004562
4. Cahoon, L.A. and Seifert, H.S., *Science*, 2009, vol. 325, pp. 764–767. 325/5941/764 [pii] doi 10.1126/science.1175653
5. Metifiot, M., Amrane, S., Litvak, S., and Andreola, M.L., *Nucleic Acids Res.*, 2014, vol. 42, pp. 12352–12366. gku999 [pii] doi 10.1093/nar/gku999
6. Javadekar, S.M. and Raghavan, S.C., *FEBS J.*, 2015, vol. 282, pp. 2627–2645. doi 10.1111/febs.13311
7. Sekridova, A.V., Varizhuk, A.M., Tatarinova, O.N., Severov, V.V., Barinov, N.A., Smirnov, I.P., Lazarev, V.N., Klinov, D.V., and Pozmogova, G.E., *Biomed. Khim.*, 2016, vol. 62, pp. 535–543.
8. Kriegs, J.O., Churakov, G., Jurka, J., Brosius, J., and Schmitz, J., *Trends Genet.*, 2007, vol. 23, pp. 158–161. S0168-9525(07)00037-6 [pii] doi 10.1016/j.tig.2007.02.002
9. Batzer, M.A. and Deininger, P.L., *Nat. Rev. Genet.*, 2002, vol. 3, pp. 370–379. nrg798 [pii] doi 10.1038/nrg798
10. Luk'yanova, T.A., Zaitseva, M.A., Karpov, V.A., and Pozmogova, G.E., *Bioorgan. Khim.*, 2008, vol. 34, pp. 83–88. doi 10.1007/s11171-008-1010-6
11. Ackermann, D., Rasched, G., Verma, S., Schmidt, T.L., Heckel, A., and Famulok, M., *Chem. Commun.* (Camb.), 2010, vol. 46, pp. 4154–4156. doi 10.1039/c0cc00390e
12. Besschetnova, I.A., Pozmogova, G.E., Chuvilin, A.N., Shchelkina, A.K., and Borisova, O.F., *Mol. Biol.* (Moscow), 2006, vol. 40, pp. 489–496.
13. Abu-Ghazalah, R.M., Irizar, J., Helmy, A.S., and Macgregor, R.B., Jr., *Biophys. Chem.*, 2013, vol. 147, pp. 123–129. S0301-4622(10)00004-9 [pii] doi 10.1016/j.bpc.2010.01.003
14. Konkel, M.K. and Batzer, M.A., *Semin. Cancer Biol.*, 2010, vol. 20, pp. 211–221. S1044-579X(10)00009-X [pii] doi 10.1016/j.semcancer.2010.03.001
15. Do, N.Q., Lim, K.W., Teo, M.H., Heddi, B., and Phan, A.T., *Nucleic Acids Res.*, 2011, vol. 39, pp. 9448–9457. gkr539 [pii] doi 10.1093/nar/gkr539
16. Tothova, P., Krafcikova, P., and Viglasky, V., *Biochemistry*, 2014, vol. 53, pp. 7013–7027. doi 10.1021/bi500773c

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