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Conformational Polymorphism and Extensibility of DNA Quadruplexes Formed by $d(GT)_n$ Repeats

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Abstract—We showed earlier that oligonucleotides 3'-$d(GT)_5$-$pO(CH_2CH_2O)_3p$-$d(GT)_5$-3' form bimolecular quadruplexes with parallel orientation of their strands, which are held by guanine quartets alternating with unpaired thymines (GT quadruplex). This work deals with the conformational polymorphism and extensibility of G quadruplexes in complex with molecules of an intercalating agent ethidium bromide (EtBr). A cooperative mechanism of EtBr binding to the GT quadruplex was revealed. The binding constant $K = (3.3 \pm 0.1) \cdot 10^4$ M^{-1}, cooperativity coefficient $\omega = 2.5 \pm 0.2$, and maximal amount of EtBr molecules intercalated in GT quadruplex ($N = 8$) were determined. It was proved experimentally by analysis of adsorption isotherms and theoretically by mathematical modeling that the GT quadruplex is capable of double extension, which is indicative of the high elasticity of this four-stranded helix. Two most stable conformations of GT quadruplexes with thymine residues intercalated and/or turned outside were found by mechanico-mathematical modeling. The equilibrium is shifted toward the conformation with the looped out thymine residues upon intercalation of EtBr molecules into the GT quadruplex.

Key words: $(GT)_n$ telomeric repeats; GT and G quadruplexes of DNA, EtBr adsorption isotherms, binding constants, stoichiometry

INTRODUCTION

Telomeres are located at the ends of eukaryotic chromosomes and carry out some important functions in the genome [1–14]. One strand of telomeric DNA is enriched in guanines. The single-stranded 3'-protrusion of telomeric DNA, containing G_nT_m ($n \geq 3$) repeats, is able to form *in vitro* four-stranded structures (G quadruplexes) [1–7, 15–24].

Data in favor of an important biological role of G quadruplexes were obtained. G quadruplexes were shown to inhibit telomerase, which participates in the regulation of the length of telomeric DNA [2–7, 25, 26]. A number of regulatory proteins specifically binding G quadruplexes of DNA were found [4, 5, 8–13, 27].

G quadruplexes with the block arrangement of guanines (G_n, $n \geq 3$) in the DNA strand appear to be structurally polymorphous. Four G-rich strands form quadruplexes with the parallel strand orientation, G4-DNA [1–5]. Dimerization of two hairpin structures or folding of one strand on itself result in formation of G quadruplexes with antiparallel strands [2–6, 22–24, 28–30].

PAGE in native conditions has shown that oligonucleotides formed by simple $d(GT)_n$ repeats, appearing with a high probability both in telomeres and in other regulatory regions of the genome, do not form duplex structures but rather exist as single-stranded structures [29, 30]. However, our investigations of oligonucleotides joined by a neutral hexa- or oxyhexamethylene linker L, 3'-$d(GT)_5$-L-$d(GT)_5$-3', have shown for the first time the possibility of *in vitro* formation of intermolecular quadruplexes. Four $d(GT)_5$ strands in these quadruplexes are parallel to each other and are fixed by cyclic guanine quartets alternating with unpaired thymines turned outside [17, 31–33]. For short, these structures are named GT quadruplexes of DNA.

The interaction of intercalating ligands with G quadruplexes was studied [17, 25–28, 31, 34–40] and it was shown that parallel and antiparallel G quadruplexes with guanine blocks bind EtBr, porphyrins, and other ligands mainly on the surface [25, 26, 34–40]. By contrast to G4-DNA, our preliminary studies of EtBr complex formation with GT quadruplexes joined by hexamethylene linkers suggest intercalation of EtBr [31, 32].

In this work we continued the investigation of EtBr complexes with GT quadruplexes formed by 3'-

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$d(GT)_5$-L-$d(GT)_5$-3' with oxyhexamethylene linkers. Based on analysis of adsorption isotherms, the cooperative mechanism of the ligand binding to GT quadruplex was established. The binding constants and the number of binding sites were determined. The GT quadruplex structure was shown to be highly elastic and capable of double extension upon EtBr intercalation.

EXPERIMENTAL

Preparations. Linked oligonucleotides 3'-$d(GT)_5$-$pO(CH_2CH_2O)_3p$-$d(GT)_5$-3' (parGT) and 5'-d(GTACTCCCAT)-$pO(CH_2CH_2O)_3p$-d(ATGGGAG-TAC)-3' (duplex I, taken for comparison) were synthesized by the method described in detail previously [41]. EtBr was from Serva.

Samples were dissolved in 0.01 M Na-phosphate buffer, pH 7.0, 0.1 M NaCl. Temperature was kept constant at 3°C.

The concentration of oligonucleotides was determined by UV spectra. Molar extinction coefficients are given in moles of nucleotides at 90°C: $\varepsilon_{260} = 10{,}550\ M^{-1}\ cm^{-1}$ for parGT [17] and $\varepsilon_{260} = 10{,}300\ M^{-1}\ cm^{-1}$ for duplex I [42]. The concentration of oligonucleotides in solution was kept constant and equal to $2 \cdot 10^{-5}$ M. Solutions containing EtBr were prepared in the same Na-phosphate buffer taking its molar extinction coefficient to be equal to $\varepsilon_{485} = 5600\ M^{-1}\ cm^{-1}$ [41].

Fluorescence intensity and spectra were registered on a spectrofluorimeter Aminco SPF-1000 in the constant-temperature cells.

The fluorescence quantum yield (*q*) was calculated using the fluorescence and adsorption spectra of dyes adsorbed on oligonucleotides in comparison with those upon dye adsorption on DNA [41].

The time of EtBr fluorescence (τ) was measured on a phase fluorometer [43]. Measurements were carried out for excitation at $\lambda = 546$ nm, fluorescence was registered at $\lambda > 600$ nm.

Isotherms of EtBr adsorption. The concentration of the nucleic acid-bound EtBr was calculated using a fluorimetric method [41, 44]:

$$C_2/C_0 = (I - I_1)/(I_2 - I_1), \quad (1)$$

where I, I_1, and I_2 are the measured fluorescence intensities of a sample under study, control samples with free and fully bound dye, respectively; $C_0 = C_1 + C_2$ is the sum of concentrations of free (C_1) and bound (C_2) dye. Parameter $r = C_2/P$ corresponded to the bound dye concentration related to that of base pairs with the dimension of mol/mol.

For the EtBr anticooperative binding to DNA, the following equation was used [45, 46]:

$$r/C_1 = K(1 - nr)[(1 - nr)/1 - nr + r]^{n-1}, \quad (2)$$

where K is the constant of ligand binding to an isolated site; n is the number of base pairs necessary for binding of one ligand molecule.

Curves of cooperative type of binding were analyzed using equations of McGhee and von Hippel [47]:

$$\begin{aligned} r/c_1 = {} & K(1 - nr)([(2\omega - 1)(1 - nr) \\ & + (r - R)]/[2(\omega - 1)(1 - nr)])^{n-1} \\ & \times ([1 - (n - r)r + R]/[2(1 - nr)])^2, \end{aligned} \quad (3)$$

where $R = [1 - (n + 1)r]^2 + 4\omega r(1 - nr)^{1/2}$; ω is the cooperative binding parameter.

Molecular mechanics modeling of conformational states of GT quadruplexes and their intercalation complexes with EtBr molecules. Conformational analysis of both an octameric fragment of GT quadruplex of four parallel $d(GT)_4$ strands fixed by four G quartets and intercalation complexes of GT quadruplex with EtBr molecules was carried out using the Hyper Chem™ 5.02 program for a molecular mechanics model of the potential energy calculation and AMBER 3 parameters. The calculations were carried out for two molecular models of the GT quadruplex: model I, in which thymines are beyond the space of stacking with G quartets and are turned outside; and model II, where thymines of each of four strands are located in the space of stacking with G quartets. Our previous model calculations showed that these structural variants of GT quadruplexes are principally possible [31]. Starting conformations for energy optimization were designed using symmetry transformations in the Hyper Chem™ 5.02 interactive mode. To construct the EtBr intercalation complexes with GT quadruplex, noncanonical oligonucleotides obtained in advance by a special program were used. In the intercalation complex, starting conformation correlated with model II, the distance between guanine and thymine quartets was increased by 3.4 Å for intercalation of two EtBr cations. In the starting conformation corresponding to model I, both EtBr cations were placed between two neighboring guanine quartets into the stacking space that is free because the thymines are turned outside. For the two models, the potential energy of complexes was optimized many times, changing the starting position of EtBr cations.

Atomic charges on the EtBr cation were obtained by a semiempirical method AM1. In the calculation of electrostatic interactions, dielectric constant was taken to be in direct proportion to the distance (with the coefficient of proportionality equal to one), the contribution of 1–4 interactions was considered with the weight of 0.5. The minimization of potential energy was carried out using the Pollack–Ribjerre algorithm up to a gradient of 10^{-4}.

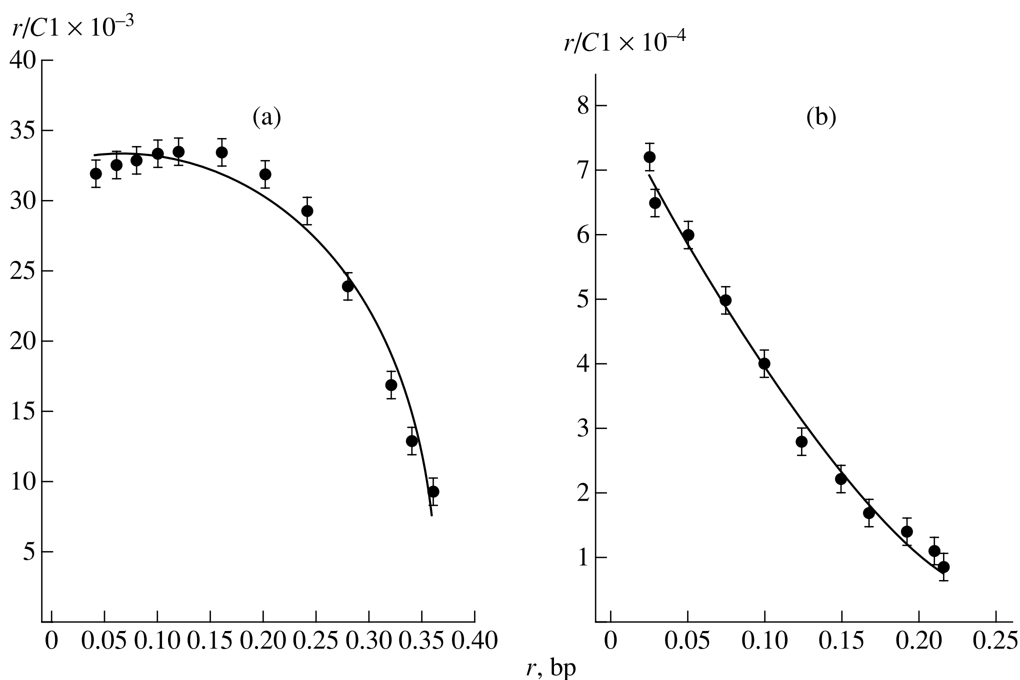


Fig. 1. Isotherms of EtBr adsorption on GT quadruplex (a) and duplex I (b) registered by a fluorimetric method in 0.01 M Na-phosphate buffer, pH 7.0, 0.1 M NaCl, $t = 3°C$. Parameter r is given per bp.

RESULTS AND DISCUSSION

Quantum yield and time of fluorescence of EtBr complexes with GT quadruplexes and DNA. The time (τ) and quantum yield (q) of fluorescence of EtBr complexes with GT quadruplex (EtBr–GT) and duplex I (EtBr–DNA) were measured. Quantum yield $q = 0.15 \pm 0.01$ and time $\tau = 24 \pm 1$ ns for EtBr–GT complexes coincided within experimental error with $q = 0.16 \pm 0.001$ and $\tau = 25 \pm 1$ ns for EtBr–DNA complexes. The coincidence of q and τ for EtBr–GT with the same parameters for EtBr–DNA is in favor of EtBr intercalation [31, 41, 44].

EtBr sorption on GT quadruplex. Figure 1 shows the isotherms of EtBr adsorption on GT quadruplex (a) and for comparison on duplex I (b), two strands of which are joined by the same oxyhexamethylene linker as in parGT molecules forming GT quadruplex. The curves were obtained by fluorimetric titration in Na-phosphate buffer, 0.1 M NaCl at 3°C.

Table 1. Parameters of EtBr binding to GT quadruplex and DNA

Sample	$K \cdot 10^{-4}$, M^{-1}	n	N*	ω**
GT quadruplex	3.3 ± 0.1	2.7 ± 0.1	8	2.5 ± 0.2
Duplex I	8.0 ± 0.1	3.2 ± 0.1	3	

* N, maximal amount of intercalated EtBr molecules per GT quadruplex or duplex I.

** ω, coefficient of cooperative binding in (3)

This method is known to register intercalated EtBr molecules [41, 44].

It is seen in Fig. 1a that EtBr cooperatively binds to GT quadruplex. The continuous line is the theoretical curve that obeys equation (3), coincides well with the experimental data, and is described by parameters listed in Table 1. Coefficient $\omega = 2.5 \pm 0.2$ confirms the cooperative mechanism of EtBr binding to GT quadruplex. Binding constant $K = (3.3 \pm 0.1) \cdot 10^4$ M^{-1} is indicative of a high affinity of EtBr to GT quadruplex. The maximal amount of EtBr molecules intercalated in GT quadruplex ($N = 8$) was calculated using parameter $n = 2.7 \pm 0.1$ that is equal to the number of base pairs necessary for binding of one EtBr molecule [45, 46].

We showed earlier that GT quadruplex contains five G quartets and unpaired thymines turned outside [17, 31, 32]. This means that there are only four cavities between G quartets, in each of which two EtBr molecules intercalate. Intercalation of eight EtBr molecules will result in double extension of the GT quadruplex helix due to the induced drawing apart of all G quartets for 3.4 Å.

Isotherm of EtBr adsorption on duplex I. The curve of EtBr sorption on duplex I (Fig. 1b) is well described by equation (2) derived for anticooperative binding [45, 46]. Table 1 shows $K = (8 \pm 0.1) \cdot 10^4$ M^{-1}, $n = 3.2 \pm 0.1$, and maximal amount of EtBr molecules intercalated in a decaduplex, $N = 3$. The binding parameters calculated by the method of the theoretical curve approximation to experimental correlate well

Table 2. Intramolecular potential energy of hexameric fragment of GT quadruplex of DNA

Model I with thymine residues turned outside (kcal/mol)						
E_{tot}	E_b	E_{sph}	E_{b-sph}	E_G	E_T	$E_{G,T}$
–626.5	–786.3	462.7	–302.9	–564.9	–213.1	–8.0
Model II with thymine residues intercalated between G quartets (kcal/mol)						
E_{tot}	E_b	E_{sph}	E_{b-sph}	E_G	E_T	$E_{G,T}$
–680.9	–1046.0	369.6	–4.5	–555.4	–302.8	–187.8

Note: $E_{tot} = (E_b + E_{sph} + E_{b-sph})$, value of total intramolecular potential energy of hexameric fragment of parallel GT quadruplex and its components: E_{sph}, energy of interactions of the sugar-phosphate atoms; E_{b-sph}, energy of interaction of the base atoms with those of sugar-phosphates; $E_b = (E_G + E_T + E_{G,T})$, energy of interaction between atoms of nucleic bases; E_G, energy of interaction of guanines; E_T, energy of interaction of thymines; $E_{G,T}$, energy of the guanine interactions with thymines.

with the earlier results obtained for EtBr–DNA complexes [42, 45]. Therefore, it can be stated that oxyhexamethylene linker has no effect on EtBr binding both to DNA and GT quadruplex.

Conformational states of GT quadruplexes and their intercalation complexes with EtBr molecules as shown by molecular mechanics modeling. Table 2 shows the results of optimization of potential energy of conformational models of GT quadruplexes containing thymine residues free of intercalating agent and turned outside (I) or intercalated between G quartets (II).

All values of energy components fall on the internal hexameric fragment of an octamer. This enables maximal elimination of inaccuracy caused by “end effects.” In addition to the total intramolecular potential energy, its separate components are also shown. Their comparison in conformational models I and II helps in predicting the type of structural rearrangements that may be caused by external factors.

For GT quadruplexes free of intercalating agent, a compact molecular model II with intercalated thymine residues is more favorable in energy than model I in which thymines are turned outside from the stacking space. On conversion to the monomeric unit of the four-stranded helix, the benefit is about 18 kcal/mol. Stacking energy in model II is very high (E_b = –1046.0 kcal/mol) and interactions between atoms of bases and sugar-phosphate chains are extremely low ($E_{b,\,sph}$ = –4.5 kcal/mol). The structure rigidity is increased at the expense of formation of hydrogen-bonded thymine quartets which were absent from the starting conformation. Hydrogen bonds are established during energy optimization between atomic groups N3-H and O4. Thymine quartets of the same geometry were found during analysis of NMR spectrum of a parallel G-quadruplex formed by telomeric repeats $[d(TGGTGGC)]_4$ from *Saccharomyces cerevisiae* [48]. Our calculations show that formation of the thymine quartet does not distort the geometry of four-stranded helix of GT quadruplex and retains anti-orientation of thymines.

Obtained data show that parallel GT quadruplexes have the same compact structure with stacking of the base quartets as G quadruplexes. Nevertheless, GT quadruplexes differ in principle from G quadruplexes by their ability to undergo conformational rearrangements in response to external factors such as the intercalating agents. This is explained by the fact that interaction between alternating guanine and thymine quartets, which determines structural cooperativity, is weaker than in G quadruplexes with guanine blocks in the strand (G_n, $n \geq 3$).

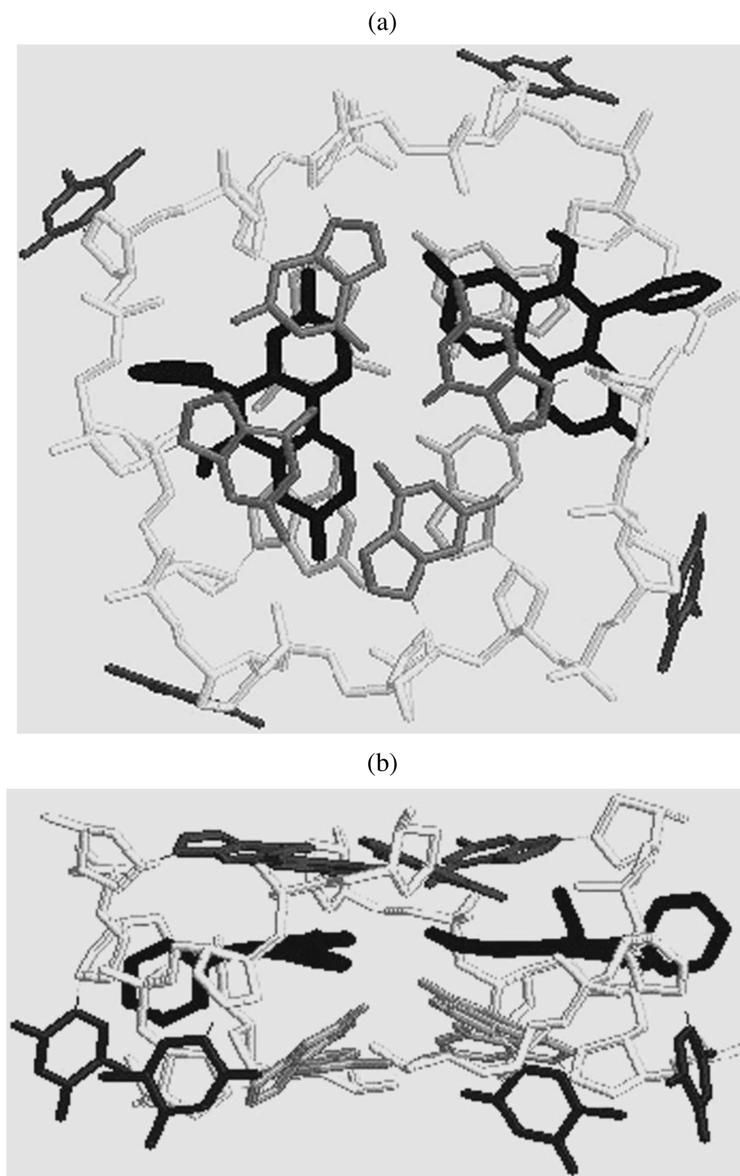
Intercalation of EtBr basically changes the conformational state of GT quadruplex. Table 3 shows that total energy of an intercalation complex with two cations of EtBr is lower if thymine bases in the struc-

Table 3. Energy of complexes consisting of the hexameric fragment of GT quadruplex of DNA and EtBr cations

Model I with thymine residues turned outside (kcal/mol)				
	E_{compl}	E_{Hex}	E_{Et}	E_{Hex-Et}
+2 EtBr	–840.9	–581.3	82.3	–341.9
+4 EtBr	–964.1	–549.2	154.4	–569.3
Model II with thymine residues intercalated between G quartets (kcal/mol)				
	E_{compl}	E_{Hex}	E_{Et}	E_{Hex-Et}
+2 EtBr	–807.7	–615.1	78.4	–271.0

Note: E_{compl}, total potential energy of the EtBr–GT quadruplex complexes calculated per hexameric fragment; E_{Hex}, energy of the hexamer in a conformation acquired in response to the intercalated EtBr cations; E_{Et}, energy of a pair of EtBr cations in the mutual position acquired by them upon intercalation in the quadruplex; E_{Hex-Et} energy of interaction of atoms of the hexameric fragment with EtBr molecules.

(a)



(b)

Fig. 2. Model I for binding of two EtBr cations to GT quadruplex containing thymine residues turned outside. EtBr molecules (black) are intercalated between two neighboring layers formed by G quartets (gray). The looped-out thymines (gray) are seen at the edges. Sugar-phosphate chains are shown in light-gray. (a) Top view, (b) side view.

ture are turned outside. Analysis of components comprising total potential energy shows that this is due to more advantageous (for 170 kcal/mol) interactions between an intercalating agent and a quadruplex. This is seen when energies of interaction between atoms of the quadruplex hexameric fragment and EtBr ($E_{\text{Hex–Et}}$) in model structures I and II are compared. As a result, intercalation should change the equilibrium position of thymines in the quadruplex. Hydrogen bonds in thymine quartets are broken, they leave the stacking space, and coupled systems of EtBr cations occupy their place. In fact, previous experimental data on the structure of the GT quadruplex complex with another intercalating agent acridine orange in salt solutions completely correlate with such scheme of conformational rearrangements [17, 31–33]. Calculations point to the possibility of simultaneous intercalation of two pairs of EtBr cations in neighboring cells separated by a guanine quartet. The quadruplex interactions with intercalated EtBr cations stabilize such a structure despite a certain increase in tension in the sugar-phosphate backbone (Table 3). These data completely correlate with the above-described experimental results concerning cooperative binding of eight EtBr molecules with GT quadruplex, two molecules per each cell formed by two adjacent G quartets (Fig. 1, Table 1).

Figure 2 shows a fragment of a model structure of intercalation complex. It is seen that a pair of EtBr cat-

ions is located in a space that was freed by four thymines turned outside. The overlapping of coupled flat rings of EtBr with G quartets appears to be incomplete. It may be that exactly this event leads to the fact that the constant of EtBr binding to GT quadruplex is lower than that for DNA (Table 1).

Model calculations and experimental data on EtBr binding to GT quadruplex point to a high elasticity of the GT quadruplex structure which can be extended twice at maximal filling with EtBr molecules of all cavities formed by G quartets. In the case of low filling, a conformation with thymine residues partly intercalated between G quartets is possible. The conformation with thymines turned outside is most probable in the case of high filling of GT quadruplexes with EtBr molecules. This fully correlates with our early experiments [31, 32].

The obtained data suggest conformational polymorphism of GT quadruplexes in their complexes with EtBr.

Differences in EtBr binding to GT and G quadruplexes. It was shown previously that EtBr binds to G quadruplexes containing guanine blocks via a noncooperative mechanism. The calculated constants of EtBr binding to G quadruplexes were higher than those for DNA. The number of binding sites was no higher than one or two EtBr molecules per G quadruplex [38]. Biochemical investigations, NMR, and mechanico-mathematical modeling revealed that intercalating ligands are mainly adsorbed on the surface of G quadruplexes [25, 26, 34–40, 49].

We have already shown that EtBr binds cooperatively to the GT quadruplex (Fig. 1). Eight EtBr molecules bind to one GT quadruplex, two per each cell formed by neighboring G quartets. The constant of EtBr binding to GT quadruplex was 2.5 times lower than that calculated for EtBr–DNA complexes under the same experimental conditions. A low constant of EtBr binding to GT quadruplex can be explained by incomplete overlapping of the guanine planes with that of EtBr molecule (Fig. 2).

Analysis of data described here and in the literature on binding of EtBr and other intercalating agents with multihelical DNA structures such as G quadruplexes [25, 26, 34–40, 49] and triplexes [42, 50–53] allows us to conclude that the structure of GT quadruplex has the highest elasticity as compared with other known multihelical DNA structures. Double-stranded and recombinant triplex DNA were extended upon intercalation of EtBr and propidium no more than by 50% [42, 53]. Other known DNA triplexes did not stretch by more than 30% [43, 50–53]. Parallel and antiparallel G quadruplexes formed by the DNA strands containing guanine blocks (G_n, $n \times 3$) practically did not stretch out, and bound intercalating ligands mainly on the surface of G quadruplexes [25, 26, 34–40, 49]. Probably, moving apart two neighboring G quartets in G quadruplexes with guanine blocks requires much energy and they appear to be inaccessible for intercalating ligands. This supposition is confirmed by comparison of enthalpies of the G-quadruplex formation. We showed previously that enthalpy of the GT quadruplex formation ($\Delta H = -5.6$ kcal/mol per G quartet) [32, 33] is significantly lower as compared with enthalpies of known parallel and antiparallel G quadruplexes with guanine blocks in their strands ($-12 \le \Delta H \le -36$ kcal/mol) [3].

A possible biological role of GT and G quadruplexes. Until recently, GT and G quadruplexes have not been found *in vivo*. However, some experimental data show that certain genomic nucleotide sequences can form four-stranded structures fixed by G quartets, such as telomeres [2], immunoglobulin regions [19, 54], c-*myc* promoter [14], and others [40]. It was shown that G quadruplexes of telomeric DNA can inhibit telomerase [2–7, 25, 26, 49, 55]. Therefore, it becomes necessary to design stable G quadruplexes complexed with intercalating ligands which are able to inhibit tumor cells [2–7, 25, 26, 49, 55].

It has been shown recently that G quadruplexes of DNA specifically bind to the proteins playing an important role in intracellular processes. The yeast telomere-binding protein RAP1 [11, 12], β-subunit from *Oxitrichia* [8], and human topoisomerase I [28] not only bind to G quadruplexes but stimulate their formation. Phenylalanine substitution for tyrosine in the active center of human topoisomerase I results in elimination of the enzyme ability to stimulate formation of G quadruplexes. This result probably indicates that the topoisomerase I active center is involved in nucleation of the G-quadruplex formation [28].

The described here experiments on the EtBr interaction with GT quadruplex and theoretical analysis of the balance of forces of intramolecular interactions allow one to foresee structural alterations of this quadruplex in response to external factors. For example, it can be supposed that formation of a complex with proteins can stabilize the structure with the looped out thymines. Conformational movements in such systems are poorly coupled (dispersion and electrostatic interactions between thymines and guanines are weak), so, owing to torsion movements, thymines on the surface of GT quadruplex can easily adapt to the changed quadruplex structure emerging upon interaction with proteins. If the content of aromatic amino acid residues in proteins is high as in RAP1 [11–13], they can easily intercalate between neighboring G quartets.

CONCLUSION

Experimental data on EtBr binding to GT quadruplex obtained in this work and mechanico-mathemat-

ical models allowed us to reveal new properties of four-stranded DNA structures formed by simple $(GT)_n$ repeats, such as their high elasticity in contrast to G quadruplexes of G_nT_m repeats with guanine blocks forming rigid helices.

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REFERENCES

1. Sen, D. and Gilbert, W., *Curr. Opin. Struct. Biol.*, 1991, vol. 1, pp. 435–438.
2. Williamson, J.R., *Annu. Rev. Biophys. Biomol. Struct.*, 1994, vol. 23, pp. 703–730.
3. Pilch, D.S., Plum, G.E., and Breslauer, K.J., *Curr. Opinion Struct. Biol.*, 1995, vol. 5, pp. 334–342.
4. Venczel, E.A. and Sen, S., *J. Mol. Biol.*, 1996, vol. 257, pp. 219–224.
5. Rhodes, D. and Giraldo, R., *Curr. Opin. Struct. Biol.*, 1995, vol. 5, pp. 311–322.
6. Kang, C.H., Zhang, X., Ratliff, R., Moyzis, R., and Rich, A., *Nature*, 1992, vol. 356, pp. 126–131.
7. Wang, K.J. and Patel, D.J., *J. Mol. Biol.*, 1994, vol. 234, pp. 1177–1183.
8. Fang, G. and Cech, T.R., *Cell*, 1993, vol. 74, pp. 875–885.
9. Liu, Z. and Gilbert, W., *Cell*, 1994, vol. 77, pp. 1083–1092.
10. Liu, Z., Lee, A., and Gilbert, W., *Proc. Natl. Acad. Sci. USA*, 1995, vol. 92, pp. 6002–6006.
11. Giraldo, R. and Rhodes, D., *EMBO J.*, 1994, vol. 13, pp. 2411–2420.
12. Giraldo, R., Suzuki, M., Chapman, L., and Rhodes, D., *Proc. Natl. Acad. Sci. USA*, 1994, vol. 91, pp. 7658–7666.
13. Konig, P., Giraldo, R., Chapman, L., and Rhodes, D., *Cell*, 1996, vol. 85, pp. 125–136.
14. Simonsson, T., Pesinka, P., and Kubista, M., *Nucleic Acids Res.*, 1998, vol. 26, pp. 1167–1172.
15. Wang, Y. and Patel, D.J., *Biochemistry*, 1992, vol. 31, pp. 8112–8119.
16. Balagurumorthy, P. and Brahmachary, S.K., *J. Biol. Chem.*, 1994, vol. 269, pp. 21858–21869.
17. Borisova, O.F., Shchyolkina, A.K., Timofeev, E.N., and Florentiev, V.L., *FEBS Lett.*, 1992, vol. 306, pp. 140–142.
18. Blackburn, E.H., *Cell*, 1994, vol. 77, pp. 621–623
19. Zakian, V.A., *Science*, 1995, vol. 270, pp. 1601–1607.
20. *Telomeres*, Blackburn, E.H. and Greider, C.W., Eds., N.Y.: Cold Spring Harbor Lab. Press, 1995.
21. Aboul-elc, F., Murchie, A.I.H., and Lilley, D.M.J., *Nature*, 1992, vol. 360, pp. 280–282.
22. Aboul-elc, F., Murchie, A.I.H., Norman, D.G., and Lilley, D.M.J., *J. Mol. Biol.*, 1994, vol. 243, pp. 458–471.
23. Schultze, P., Smith, F.W., and Feigon, J., *Structure*, 1994, vol. 2, pp. 221–233.
24. Smith, F.W. and Feigon, J., *Biochemistry*, 1993, vol. 32, pp. 8682–8692.
25. Wheelhouse, R.T., Sun, D., Han, H., Han, F.X., and Hurley, L.H., *J. Am. Chem. Soc.*, 1998, vol. 120, pp. 3261–3262.
26. Fedoroff, O.Yu., Salazar, M., Han, H., Chemeris, V.V., Kervin, S.M., and Hurley, L.H., *Biochemistry*, 1998, vol. 37, pp. 12367–12374.
27. Catasty, P., Chen, X., Moyzis, R.K., Bradbury, E.M., and Gupta, G., *J. Mol. Biol.*, 1996, vol. 264, pp. 534–545.
28. Arimondo, P.B., Riou, J.-F., Mergny, J.-L., Tazi, J., Sun, J.-S., Garestier, Th., and Helene, C., *Nucleic Acids Res.*, 2000, vol. 28, pp. 4832–4838.
29. Lustig, A., *Nucleic Acids Res.*, 1992, vol. 20, pp. 3021–3028.
30. Henderson, E., Hardin, C.C., Walk, S.K., and Tinoco, I., *Cell*, 1987, vol. 51, pp. 899–908.
31. Borisova, O.F., Shchyolkina, A.K., Mamayeva, O.K., Lysov, Yu.P., Chernyi, A.A., Gorin, A.A., Timofeev, E.N., and Florentiev, V.L., *Mol. Biol.*, 1992, vol. 26, pp. 452–462.
32. Borisova, O.F., Shchyolkina, A.K., Kharitonov, V.N., Pozmogova, G.E., Livshitz, M.A., and Florentiev, V.L., *Mol. Biol.*, 1999, vol. 33, pp. 503–511.
33. Livshitz, M.A., Borisova, O.F., Mamayeva, O.K., and Shchyolkina, A.K., *J. Biomol. Struct. Dynam.*, 1999, vol. 16, p. 1314.
34. Arthanari, H., Basu,, S., Kawano T.L., and Bolton, P.H., *Nucleic Acids Res.*, 1998, vol. 26, pp. 3724–3728.
35. Chen, Q., Kuntz, I.D., and Shafer, R.H., *Proc. Natl. Acad. Sci. USA*, 1996, vol. 93, pp. 2635–2639.
36. Anantha, N.V., Azam, M., and Sheardy, R.D., *Biochemistry*, 1998, vol. 37, pp. 2709–2714.
37. Han, F.X., Wheelhouse, R.T., and Hurley, L.H., *J. Am. Chem. Soc.*, 1999, vol. 121, pp. 3561–3570.
38. Guo, Q., Lu, M., Marky, L.A., and Kallenbach, N.R., *Biochemistry*, 1992, vol. 31, pp. 2451–2455.
39. Read, M.A. and Neidle, S. J., *Biochemistry*, 2000, vol. 39, pp. 13422–13432.
40. Kervin, S.M., *Current Pharmaceutical Design*, 2000, vol. 6, pp. 441–471.
41. Borisova, O.F., Golova, Yu.B., Gottikh, B.P., Zibrov, A.S., Il'icheva, I.A., Lysov, Ju.P., Mamayeva, O.K., Chernov, B.K., Chernyi, A.A., Shchyolkina, A.K., and Florentiev, V.L., *J. Biomol. Struct. Dynam.*, 1991, vol. 8, pp. 1187–1210.
42. Shchyolkina, A.K. and Borisova, O.F., *FEBS Lett.*, 1997, vol. 49, pp. 27–31.

43. Borisova, O.F. and Tumerman, L.A., *Biofizika*, 1965, vol. 10, pp. 32–36.

44. Le Pecq, J.B. and Paoletti, C., *J. Mol. Biol.*, 1967, vol. 27, pp. 84–106.

45. Crothers, M.D., *Biopolymers*, 1968, vol. 6, pp. 575–584.

46. Zasedatelev, A.S., Gurskii, G.V., and Volkenstein, M.V., *Mol. Biol.*, 1971, vol. 5, pp. 245–252.

47. McGhee, J.D. and von Hippel, P.H., *J. Mol. Biol.*, 1974, vol. 86, pp. 469–489.

48. Patel, P.K. and Hosur, R.V., *Nucleic Acids Res.*, 1999, vol. 27, pp. 2457–2464.

49. Mergny, J.-L. and Helene, C., *Nature Med.*, 1998, vol. 12, pp. 1366–1367.

50. Waring, M.J., *Biochem. J.*, 1974, vol. 143, pp. 483–486.

51. Mergny, J.L., Collier, D., Rougee, M., *et al.*, *Nucleic Acids. Res.*, 1991, vol. 19, pp. 1521–1526.

52. Scaria, P.J. and Shafer, R.H., *J. Biol. Chem.*, 1991, vol. 266, pp. 5417–5423.

53. Borisova, O.F., Shchyolkina, A.K., Timofeev, E.N., Tsybenko, S.Ju., Mirzabekov, A.D., and Florentiev, V.L., *J. Biomol. Sruct. Dynam.*, 1995, vol. 13, pp. 15–27.

54. Chung, I.K., Mehta, V.B., Spitzner, J.R., and Muller, H.T., *Nucleic Acids Res.*, 1992, vol. 20, pp. 1973–1977.

55. Zahler, A.M., Williamson, J.R., Cech, T.R., and Prescott, D.M., *Nature*, 1991, vol. 350, pp. 718–720.