Volume 306, number 2,3, 140–142 FEBS 11277 July 1992

Evidence for the tetraplex structure of the d(GT)$_n$ repetitive sequences in solution

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Received 15 May 1992

The ability of oligonucleotides 3′-d(GT)$_5$pO(CH$_2$)$_6$Opd(GT)$_5$-5′ (anti[d(GT)]) and 3′-d(GT)$_5$pO(CH$_2$)$_6$Opd(GT)$_5$-3′ (par[d(GT)]) to form tertiary structures has been studied. Circular dichroism (CD) as well as the fluorescence of the ethidium bromide (EtBr) complexes with oligonucleotides and hydrodynamic volume measurements in solutions containing 0.01 M phosphate buffer, pH 7 and NaCl in concentrations from 0.1 M to 1 M, have been used. The data obtained in the temperature interval from 3°C to 10°C are in good agreement with the structure suggested earlier [1] where the par[d(GT)] and anti[d(GT)] form structures with four parallel strands in which layers of four G-residues alternate with unpaired bulged-out T-residues. Ethidium bromide interacts with the structure in a cooperative manner. Two ethidium bromide molecules intercalate between two layers of four G-residues.

Tetraplex; Parallel stranded DNA; d(GT)$_n$ sequence

1. INTRODUCTION

Polymorphous d(GT)$_n$/d(CA)$_n$ repeats have been found in many introns and regulatory sites of genes isolated from higher organisms [2,3]. A peculiar structure formed by (GT)$_n$ stretches may play an important role in its biological functioning. Nevertheless structural characteristics of (GT)$_n$ sequences have been insufficiently studied, particularly at the level of secondary structure of d(GT)$_n$/d(CA)$_n$ double helices.

Unlike (GT)$_n$ sequences, telomeric G$_n$T$_m$ sequences and G$_n$-stretch structures have been studied in detail. G$_n$T$_m$ and G$_n$ sequences are known to form G4-DNA structures with layers of four hydrogen-bounded guanine residues [4–8]. Structures with all four strands being parallel, as well as those with two back-folded and two antiparallel strands, can be formed.

In this study we present the experimental evidence for the existence of the structural organization of the alternating d(GT)$_n$ sequences in solution we suggested earlier [1].

2. MATERIALS AND METHODS

Oligonucleotides 3′-d(GT)$_5$pO(CH$_2$)$_6$Opd(GT)$_5$-5′ (anti[d(GT)]) and 3′-d(GT)$_5$pO(CH$_2$)$_6$Opd(GT)$_5$-3′ (par[d(GT)]) were synthesized as has been described before [9]. The oligonucleotide concentrations were determined from UV-spectra assuming the extinction coefficient ε_{260} at 90°C to be equal to 10,550 $M^{-1}\cdot cm^{-1}$ (in mols of nucleotides).

CD spectra were recorded using a Jobin Yvon Mark III (France) dichrograph in thermostatic cells at temperatures ranging from 0 to 80°C.

The fluorescence polarization and intensity were monitored with an AMINCO SPF 1000 (USA) spectrofluorimeter in thermostatic cuvettes at temperatures from 3 to 10°C.

The rotational relaxation time ρ and the hydrodynamic volume V of par[d(GT)] and anti[d(GT)] were calculated by measuring the fluorescence polarization P of EtBr:

$$\rho = 3\tau(1/P_o - 1/3)/(1/P - 1/P_o), \quad (1)$$

where τ is the time of fluorescence quenching (τ = 25 ns at 3°C), P_o is the limit value of P at $T/\eta \to 0$ (P_o = 42%), η is the viscosity of the solution, T is the absolute temperature [9].

The hydrodynamic volumes were calculated from the equation:

$$V = kT\rho/3\eta, \quad (2)$$

where k is the Boltzmann constant.

The unpaired nucleotides content ($1-\theta$) was determined by measuring the mean life time of acridine orange (AO) heterogeneous fluorescence registered with the phase fluorometer [1].

3. RESULTS AND DISCUSSION

The par[d(GT)] and anti[d(GT)] structures stabilized at 0–3°C remained practically native in the temperature region from 0 to ~10°C, as revealed by thermal denaturation studies [1].

The EtBr was shown to intercalate into par[d(GT)] and anti[d(GT)], the fluorescence quantum yield of EtBr bound to oligonucleotides being approximately equal to that of EtBr complexed with the thymus DNA [1,9].

Abbreviations: anti[d(GT)], 3′-d(GT)$_5$pO(CH$_2$)$_6$Opd(GT)$_5$-5′; par[d(GT)], 3′-d(GT)$_5$pO(CH$_2$)$_6$Opd(GT)$_5$-3′; anti[d(A-T)], 3′-(dA)$_{10}$pO(CH$_2$)$_6$Op(dT)$_{10}$-5′; EtBr, ethidium bromide.

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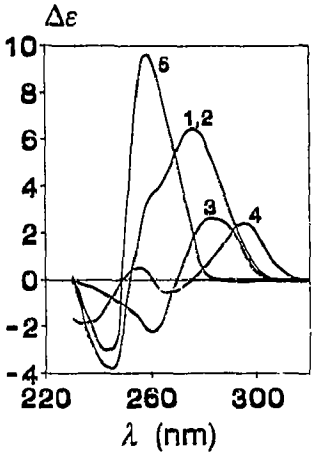

Fig. 1. CD spectra of par[d(GT)] (1) and anti[d(GT)] (2) at 3°C, par[d(GT)] and anti[d(GT)] at 80°C (3) and $d(TTAGGG)_4$ at 6°C (4). Concentration of oligonucleotides, $2 \cdot 10^{-5}$ M, 0.1 M NaCl, pH 7.5. CD spectrum of $d(G_4T_4G_4)$ in 2 μM cacodylate buffer, 0.1 μM EDTA, 70 μM NaCl at 22°C. Oligonucleotide strand concentration, 17 μM (Brahmachari S.K., personal communication).

The comparison of oligonucleotide ρ values with those for a double helix of d(CCGGATCCGG) and tetraplex of 3'-$(dA)_{10}pO(CH_2)_6Op(dT)_{10}$-5' (anti[d(A-T)]) [9] leads to conclusion that par[d(GT)] and anti[d(GT)] form a tetraplex structure in our experimental conditions (Table I).

The V values calculated using the cylinder models for a duplex and tetraplex testify to the tetraplex structure of par[d(GT)] and anti[d(GT)] (Table I). The similarity of the CD spectra of par[d(GT)] and anti[d(GT)] indicates their structural identity at 3°C (Fig. 1, curves 1 and 2). To distinguish between parallel orientation of the strands and two and two antiparallel ones, the CD spectra should be compared with those for G4-DNA of different strand orientations (S.K. Brahmachari, personal communication) (Fig. 1, curves 1–5). G4-DNA with all parallel strand formations is characterized by the increase of a positive CD band at 260 nm magnitude (Fig. 1, curve 5). The back-folded two and two antiparallel tetraplexes formed by telomeric sequences have revealed the positive band at 295 nm (Fig. 1, curve 4). The increase in the CD band magnitude at 260 nm and the absence of a CD band at 295 nm testifies to the formation of tetraplex structures with the layers of four G-residues with parallel strand orientation. Taking into account the flexibility of the hexamethylen linker [9] such a tetraplex could be constructed either of four oligonucleotide molecules or of two back-folded oligonucleotide molecules (Fig. 2).

Table I

The rotation relaxation time (ρ) and hydrodynamic volume (V) of oligonucleotides

Samples	ρ/ρ_o[a] (3°C)	ρ/ρ_o[a] (10°C)	V/V_o
Self-complementary duplex d(CCGGATCCGG)$_2$ [9]	1.0	1.0	1.0[b]
Tetraplex anti[d(A-T)]$_4$ [9]	1.6 ± 0.05	1.6 ± 0.05	1.6[b]
par[d(GT)]	–	1.55 ± 0.05	1.55[c]
anti[d(GT)]	1.6 ± 0.05	1.75 ± 0.05	1.75[c]

[a]ρ_o is the rotation relaxation time of the duplex at the same temperature (ρ_o = 7.4 ns at 3°C and 14.0 ns at 10°C); [b]V and V_o are the hydrodynamic volumes of duplex and tetraplex, respectively, which were calculated for the cylindrical model [1]; and [c]according equation (2).

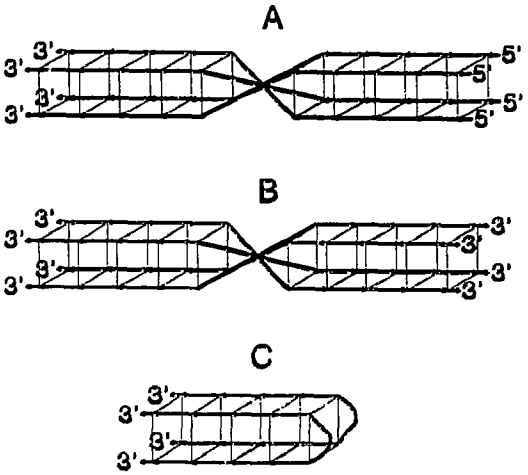

Fig. 2. Scheme of the strands orientation of anti[d(GT)] (A) and par[d(GT)] (B and C).

We have determined using acridine orange dye dimerization on unpaired nucleotides, that ~50% of all bases apparently thymines in par[d(GT)] and anti[d(GT)] are unpaired (Table II).

A striking peculiarity of EtBr binding with par[d(GT)] and anti[d(GT)] [1] is its cooperativity (Fig. 3) as has been shown for tetraplexes anti[d(A-T)] in our previous work [9]. Intercalation of the first EtBr mole-

Table II

The heterogeneous fluorescence lifetime (τ) of AO complexes with oligonucleotides

Complex with	τ_m[a] (ns)	τ_k[a] (ns)	θ[b]
par[d(GT)]	4.2 ± 0.03	7.0 ± 0.03	0.50 ± 0.06
anti[d(GT)]	3.9 ± 0.03	7.1 ± 0.03	0.57 ± 0.06
$d(TTAGGGG)_4$	3.3 ± 0.03	4.4 ± 0.03	0.80 ± 0.06
anti[d(A-T)]$_4$	4.0 ± 0.03	4.1 ± 0.03	0.98 ± 0.06

[a]τ_m and τ_k are the AO fluorescence lifetimes in the spectrum region of λ<520 nm and λ>600 nm, respectively; [b]θ, paired nucleotide fraction calculated from the equation:

$$(1-\theta)=(1-\theta)_{st}\cdot(\tau_k-\tau_m)/(\tau_k-\tau_m)_{st}$$

where the parameters without subscripts describe the oligonucleotides under investigation and those with subscript 'st' represent a nucleic acid with known fraction of unpaired nucleotides (for instance, telomeric sequence or $tRNA^{Phe}$ [1]).

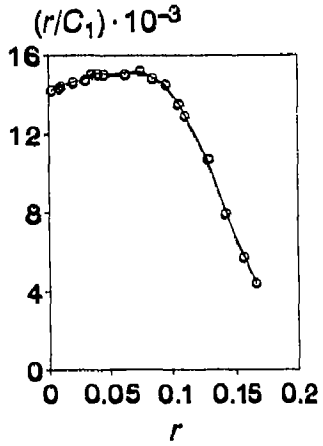

Fig. 3. The binding isotherm of EtBr on par[d(GT)] at 3°C. Concentration of nucleotides, $2\cdot10^{-5}$ M, 0.1 M NaCl, pH 7.

cule into the four-stranded helix is energetically disadvantageous and characterized by a low binding constant. In this respect it differs from intercalation of the first EtBr molecule into a short duplex [9] or triplex [11].

The unusually large number of dye binding sites observed on par[d(GT)] (r_{max} = 0.20 ± 0.01) (Fig. 3) different from that for double-stranded helices (r_{max} = 0.15 ± 0.01) allows us to suppose that two dye molecules intercalate between two adjacent layers of four G-residues.

The total combination of data obtained leads to the conclusion that oligonucleotides par[d(GT)] and anti[d(GT)] can form intermolecular tetraplexes with four parallel strands in which layers of four G-residues alterante with the unpaired bulged-out T-residues.

Acknowledgement: We thank prof. V.I. Ivanov for helpful discussion.

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