Volume 322, number 3, 304–306 FEBS 12459 May 1993

Relative stability of AT and GC pairs in parallel DNA duplex formed by a natural sequence

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Received 15 March 1993

The low-cooperative melting of parallel DNA formed by a natural 40 bp long sequence from *Drosophila*:

5′-d(TGATTGATCGATTGTTTGCATGCACACGTTTTTGTGAGCG)-3′
5′-d(ACTAACTAGCTAACAAACGTACGTGTGCAAAAACACTCGC)-3′

that possesses a normal nucleotide content was studied by using the special method of measuring the fluorescence of its complex with acriflavine as well as by conventional thermal denaturation. Acriflavine allows discrimination of the melting of AT and GC pairs because its fluorescence is quenched by neighbouring G bases. We have observed that about 40% of AT pairs melt at 14°C while the remainder melt at 42°C. The GC pairs remain stable up to ~ 40°C and melt at 54°C. The higher stability of GC pairs suggests the formation of *cis* Watson-Crick pairs in parallel DNA.

Parallel stranded DNA; Stability of GC pairs

1. INTRODUCTION

The physical capability of DNA molecules to form a parallel double helix was demonstrated recently by in vitro experiments with artificial sequences containing only AT pairs [1,2] as well as with sequences corresponding to natural DNA that also possess GC pairs [3]. The parallel double-stranded DNA containing GC pairs is characterized by low-cooperative melting [3,4]. A GC pair included into AT containing oligoduplex destabilizes parallel double helix [4]. These indirect data suggested that in parallel doubt helix, *trans* Crick-Watson GC pairs are formed possessing only one H-bond [4]. Recently the parameters of parallel double helix containing alternating oligo(A) and oligo(T) stretches were determined by scanning tunnelling microscopy [5]. These data demonstrated that parallel AT-containing DNA has two grooves of similar size. This fact is consistent with the model of Rippe et al. [4], suggesting *trans*-AT pairs in parallel-stranded DNA.

The GC pairing in parallel DNA remains more controversial. The parallel complementary probes of normal nucleotide content can be used for molecular hybridization experiments, suggesting the stability of GC-containing parallel DNA [6]. Thus, more direct experimental evidences are needed for understanding the GC-pairing in parallel DNA.

The present study describes an approach elucidating the GC-pairing by measuring the fluorescence yield of DNA-acriflavine (AF) complexes. Using this method, we observed high stability of GC pairs in parallel double helix.

2. MATERIALS AND METHODS

The decanucleotides 5′-d(GATATCCCTA)-3′ (**I**), 3′-d(CTATAGGGAT)-5′ (**II**), 5′-d(CTATAGGGAT)-3′ (**III**) and the 40-oligonucleotides 5′-d(TGATTGATCGATTGTTTGCATGCACACGTTTTTGTGAGCG)-3′ from *Drosophila* (**IV**), 3′-d(ACTAACTAGCTAACAAACGTACGTGTGCAAAAACACTCGC)-5′ (**V**), 5′-d(ACTAACTAGCTAACAAACGTACGTGTGCAAAAACACTCGC)-3′ (**VI**) were specially synthesized to obtain antiparallel (**I–II**), parallel (**I–III**) decaduplexes and antiparallel (**IV–V**), parallel (**IV–VI**) 40-duplexes [6].

The measured value of the fluorescence quantum yield (q) of AF-oligonucleotide complexes was calculated as described elsewhere [7]. According to [7] it may be expressed by the following equation:

$$q = q_{AT} \cdot n_f/(n_f + n_q) \quad (1)$$

where $n = (n_f + n_q)$ is the total number of sites for AF intercalation, n_f is a number of fluorescing sites, n_q is the number of sites where the AF-fluorescence has been quenched. The q_{AT} is the fluorescence quantum yield of AF being intercalated between two AT pairs. The concentration of bound dye was calculated using two independent methods of polarized fluorescence and absorption spectra of AF-oligonucleotide complexes [7,8].

The unpaired nucleotide fraction ($1-\theta$) was determined by measuring the fluorescence life time (τ) of the acridine orange (AO)–oligonucleotide complexes ('τ-method') [9] as well as by thermal denaturation profiles.

The times of rotational relaxation (ρ) of oligoduplexes were measured as described earlier [6,10].

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Abbreviations: AF, acriflavine; AO, acridine orange.

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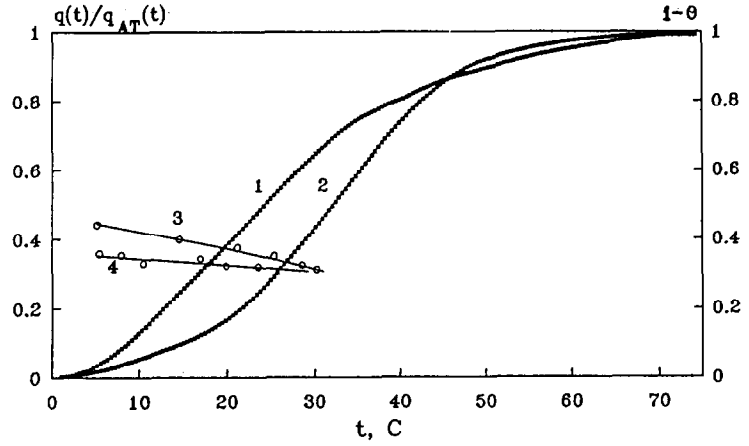

Fig. 1. The thermal denaturation curves $(1-\theta)$ of parallel duplexes **I–III** (1) and antiparallel **I–II** (2) (right ordinate). The temperature dependence of fluorescence quantum yield of AF-oligonucleotide complexes $q/q_{AT} = n_f/(n_f + n_q)$ for antiparallel duplex **I–II** (3) and parallel duplex **I–III** (4) (left ordinate). Oligonucleotide concentration was 2×10^{-4} M(N); 0.01 M phosphate buffer, pH 7, 0.25 M NaCl; AF concentration was 10^{-6} M.

3. RESULTS AND DISCUSSION

3.1. *Decaduplexes*

The possibility of antiparallel 7-nucleotide duplex formation of **(I)** and **(III)** strands instead of parallel decanucleotide formation was excluded by the annealing procedure [6]. The specialized fluorescent techniques [7,9] permitted us to check the formation of perfect antiparallel **I–II** and parallel **I–III** decaduplexes. The following data testify to the **I–III** parallel decaduplex formation: (i) the number of sites available for intercalation ($n = 9$) at 3°C was shown to be the same for both antiparallel and parallel duplexes, indicating the absence of unpaired bases in these oligonucleotides; (ii) the measured ρ values for **(I–II)** duplex and **(I–III)** duplex were the same ($\rho_{5°C} = 20 \pm 1$, ns), indicating that the hydrodynamic volumes of the duplexes are equal to each other; (iii) by the 'τ-method' the $(1-\theta)$ value at 5°C was shown to be equal to zero for the antiparallel decaduplex and 0.07 ± 0.02 for the parallel decaduplex indicating the presence of one unstable terminal base pair in the latter.

The melting of antiparallel and parallel decaduplexes is highly cooperative and proceeds by the 'all-or-none' principle. As a result the $q(t)$ values of AF-decanucleotide complexes are not markedly affected by temperature (Fig. 1). The ratio $q_{5°C}/q_{AT(5°C)}$ for antiparallel decaduplex was determined to be equal to 0.44 ± 0.02. It is in good agreement with theoretically calculated value: $q/q_{AT} = n_f/n_f + n_q = 4/9 = 0.445$. However, the observed $q_{5°C}/q_{AT(5°C)}$ for parallel decaduplex is equal to $n_f/n_f + n_q = 3/8 = 0.34 \pm 0.02$ (Fig. 1) and apparently accounts for the unstable terminal AT pair (but not terminal GC pair). In the case of unstable terminal GC pair, the theoretical value q/q_{AT} would be equal to 0.50.

The mean values of Vant-Goff enthalpy of melting were calculated to be equal to ~ -24.7 kcal/mol for parallel decaduplex and ~ -35.3 kcal/mol for antiparallel decaduplex using the method [11].

3.2. *40-nucleotide duplexes*

The measured times of rotational relaxation of AF and ethidium bromide complexes with **(IV–V)** and **(IV–VI)** oligonucleotides are the same ($\rho_{5°C} = 88 \pm 4$ ns) and testify to the absence of hairpins or other unusual structures in the parallel 40-nucleotide duplex [6]. The absence of mismatches and unpaired nucleotides in parallel 40-duplex was checked by the 'τ-method' [9]. The measured fraction of unpaired nucleotides $(1-\theta)$ at 5°C for parallel stranded **(IV–VI)** 40-duplex did not exceed 0.08 ± 0.03 indicating the perfect parallel 40-duplex formation. These data are in good agreement with the measurements of the number of sites available for intercalation ($n = 39$) in **(IV–V)** [6]. Theoretically calculated by Eqn. 1 for $n = 39$ the AF fluorescence quantum yields of the parallel and antiparallel 40-nucleotides are the same and correlate with the experimental values $q/q_{AT} = 0.34 \pm 0.01$ at 5°C. This is in good agreement with our finding that both oligonucleotides are perfect duplexes ($n_f/n_f + n_q = 0.31$). In addition, molecular hybridization experiments support the perfect parallel 40-nucleotide **(IV–VI)** duplex formation [6].

For parallel 40-duplex the unpaired nucleotide fraction $(1-\theta)$ defined by the thermal denaturation curve (Fig. 2) matches that determined by the curve 2, which reflects the decrease in AT pairs. It should be noted that the independent melting of AT base pairs by itself in parallel 40-nucleotide sequence testifies to a strikingly low cooperativity of this duplex melting process. The width of this duplex melting curve (Fig. 2) correlates well with these data. This feature of a parallel helix containing GC pairs may play an important biological role in its interaction with ligands. The fraction of paired bases θ determined by hyperchromicity at $t > T_m$

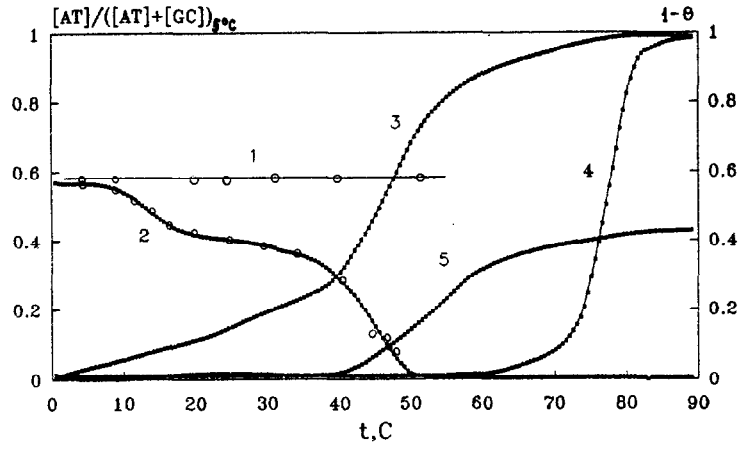

Fig. 2. The temperature dependence of the AT pairs fraction {[AT]/([AT] + [GC])$_{5°C}$} of the AF-40-nucleotide complexes (left ordinate). 1 = antiparallel duplex **IV–V**; 2 = parallel duplex **IV–VI**. 3,4 (right ordinate) = thermal denaturation curves ($1-\theta$) of parallel **IV–VI** and antiparallel – **IV–V** 40-duplex, respectively. 5 = the melting curve of GC pairs calculated by subtraction from the curve (3) of the contribution of melted AT pairs estimated with the help of curve (2). Oligonucleotide concentration was 2.4×10^{-4} M(N); 0.01 M phosphate buffer, pH 7, 0.25 M NaCl; AF concentration was 10^{-6} M. [AT]/([AT] + [GC])$_{5°C}$ = $\theta(q/q_{AT})^{1/2}$, where [AT] is the AT pair concentration in the 40-nucleotide studied in dependence on temperature, ([AT] + [GC])$_{5°C}$ is the total base pairs concentration at 5°C in the 40-nucleotide [7].

is mainly correlated with unmelted GC pairs (Fig. 2, curve 5). Therefore the GC pairs are shown to be more stable than AT pairs in parallel DNA fragment formed by natural sequence **IV**.

The two-stage type of AT pair melting in parallel 40-duplex is probably due to the presence of alternating and non-alternating AT pairs of different stability as well as to AT pairs weakening at the boundaries with GC pairs. Approximately 40% of AT pairs melt at T_m = 14°C, and the rest of the AT pairs melt at about T_m = 42°C (Fig. 2, curve 2). The T_m of GC pairs lies around 53°C (Fig. 2, curve 5).

Taking into account the above experimental data and considering the AT pairs in parallel duplexes to be of *trans* Crick-Watson type [12] we suppose that in the parallel 40-duplex studied most of the GC pairs are probably of *cis* Watson-Crick type with three hydrogen bonds. In this case at the AT/GC pair boundary distortions of sugar-phosphate backbone and of base pairs stacking may occur and in particular account for high sensitivity of these sites to S1 and S7 nuclease action [4].

It should be noted that the difference in thermostability between antiparallel and parallel 40-duplexes (Fig. 2) is significantly larger than that between decaduplexes (Fig. 1) of the same GC content. It may be due to different nucleotide sequence as well as to a higher concentration of the AT/GC boundaries per base pair in the parallel 40-duplex as compared with the parallel decaduplex.

Thus, low cooperativity of melting process in the parallel 40-nucleotide duplex of natural sequence has been observed.

The GC pairs in the 40-duplex were shown to be mainly more thermostable than AT pairs.

Acknowledgements: The authors thank Prof. V.L. Florentiev, Prof. V.I. Ivanov and D.Yu. Krylov for helpful discussion; Prof. D.L. Brutlag for his help. This study was supported with the Grant N50, programme 'Cardinal Trends in Genetics' (Russia).

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