Antiparallel DNA duplex formation between alternating α d(GA)$_n$ and β d(GA)$_n$ sequences

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Abstract Alternating polypurine d(GA)$_n$ sequences exhibit a considerable polymorphism. Here we report that α d(GA)•d(GA) sequences form an antiparallel stranded duplex DNA at neutral pH. The spectroscopic, electrophoretic and thermodynamic properties of the α/β chimeric oligodeoxynucleotide, 5′-d(GA)$_4$(T)$_4$ α d(AG)$_4$T-3′, support the formation of a hairpin structure with antiparallel strands in the stem. The optical properties of this novel antiparallel structure are different from the parallel stranded homoduplex formed by d(GA)G$_7$. This α/β hairpin has a remarkably high T_m of 44.5°C in 0.4 M NaCl with a van't Hoff enthalpy comparable to that of a parallel d(GA)$_n$ duplex. Base pairing was confirmed by T$_4$ polynucleotide ligase catalyzed joining of the α/β hairpin to an antiparallel bimolecular duplex and by non-denaturing gel electrophoresis using duplexes containing sequence constraints. Both support the presence of αG-G and αA-A base pairing in the antiparallel 5′-d(GA)$_4$(T)$_4$ α d(AG)$_4$T-3′ intramolecular duplex. This study adds to the polymorphic nature of alternating d(GA)$_n$ sequences as well as providing novel homopurine base pairing approaches for probing polypurine polypyrimidine sequences.

Key words: DNA structure; Alpha anomer; GA repeat; Oligodeoxynucleotide; Hairpin

1. Introduction

Polypurine polypyrimidine sequences occur frequently in eukaryotic DNA and have been implicated in biological processes such as transcriptional regulation and recombination [1,2]. The alternating sequence d(GA)•d(TC), constituting up to 0.4% of the mammalian genome [3], has been the focus of considerable structural investigations. In addition to having the potential to form triplex H-DNA under topological stress [1,4–6], the separate strands of this duplex have unique properties themselves. Specific binding proteins for single stranded d(TC)$_n$ have been detected in nuclear extracts of mammalian species including mouse, monkey and human [7]. Polymorphic structures have been postulated for the alternating homopurine sequence d(GA)$_n$ including parallel stranded duplexes [8,9], antiparallel duplexes [10–12], tetraplex structures [10,13] and single stranded helixes [14–17]. In particular, the evidence for a parallel stranded duplex has been well documented using a variety of d(GA)$_n$ linear sequences [8]. In the proposed model, this parallel stranded d(GA) duplex contains A-A and G-G base pairs. The parallel stranded nature of the (GA) duplex has been confirmed using a (GA) strand containing hairpin structure with built in polarity reversal [18].

Parallel stranded duplex structure has also been assigned to the duplex formed from α anomeric oligodeoxynucleotides with the complementary β anomeric oligodeoxynucleotides [19,20]. The parallel stranded nature of the α/β duplexes as well as the d(GA)$_n$ duplex provided a rational to study the duplex potential of α d(GA)$_n$ sequences. Specifically we postulate that a duplex formed between α d(GA)$_n$ and d(GA)$_n$ would have an antiparallel strand orientation.

The investigation of this potential antiparallel stranded duplex was studied in a hairpin model system that could form an antiparallel α d(GA)$_n$•d(GA)$_n$ structure intramolecularly. Therefore, a chimerical deoxyoligodeoxynucleotide that contains both α d(GA)$_n$ and d(GA)$_n$ components was synthesised and its structural properties determined.

2. Materials and methods

2.1. Synthesis, purification and gel electrophoretic analysis of the oligodeoxynucleotides

The protocol for the synthesis of the 3′-(dimethoxytrityl)-5′-(2-cyanoethyl-*N*,*N*-diisopropylphosphoroamidites of α-D-deoxyadenosine and α-D-deoxyguanosine from the respective nucleosides (R.I. Chemicals) is presented elsewhere [21]. Amidine protection of the amino group was used in the protocol for α-D-dG [22]. Synthesis products were purified by denaturing 15% polyacrylamide gel (5% crosslinking) in 8 M urea in TBE buffer (90 mM Tris-borate and 5 mM EDTA, pH 8.3). Bands containing the product were eluted by diffusion and desalted on a Sephadex G-25 column. The sequences of the α and β d(GA) containing oligodeoxynucleotides used in this study are shown in Fig. 1. Purified oligodeoxynucleotides were analyzed on 20% native gels (5% crosslinking). The running buffer was 90 mM Tris-borate, 15 mM $MgCl_2$. Samples (0.2 OD units or ^{32}P labeled) were applied in 10% sucrose and run at 4°C at 10 V/cm overnight and bands were visualized by UV shadowing or by autoradiography.

2.2. Enzymatic reactions

Oligodeoxynucleotides were 5′ ^{32}P end-labeled by T$_4$ polynucleotide kinase (Pharmacia) as described previously [21]. The ligation reactions (10 μl) were incubated at 4°C overnight under standard conditions [23] with 1 unit of T$_4$ polynucleotide ligase (Boehringer Mannheim) and a 1:1 ratio of 5′ ^{32}P labeled oligodeoxynucleotide I and a 23 base oligodeoxynucleotide (Fig. 3) at 1.6 OD/ml. A heptamer was added at a six-fold excess to provide a ligatable duplex. Aliquots were applied to a denaturing polyacrylamide gel and autoradiography was used to locate the labeled products.

2.3. Optical spectroscopy

Ultraviolet (UV) absorption spectra and thermal denaturation profiles were recorded with a Cary 3E spectrophotometer equipped with thermostatted cuvette holders. The concentration of the oligodeoxynucleotides was determined from their molar extinction coefficients which were based on the sum of the absorbance of the mononucleotides at 90°C in 5 M $NaClO_4$, pH 7.2. Hyperchromicity profiles were obtained from spectra recorded at 85°C divided by spectra at 5°C. The thermal denaturation of the oligodeoxynucleotide was followed at 260 nm and the temperature was increased at 0.5°C/min. Absorbance readings were collected at 0.5°C intervals. The concentration independent melting curves of the DNA duplexes were analyzed using a

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two state model. The enthalpy of the formation of the duplex ($\Delta H°$) and the T_m were extracted from each melting curve using a six-parameter fitting routine in which the temperature dependent absorbances of the helix and coil forms are accounted for [24]. Entropies for the denaturations are calculated from

$$\Delta S° = \Delta H°_{vH}/T_m$$

Circular dichroism (CD) spectra were recorded on a Jasco J-500C spectropolarimeter, interfaced to a 386 PC and equipped with a thermostatted cuvette holder. The circular dichroism $\Delta\varepsilon$ (M^{-1} cm^{-1}) is expressed per M nucleotide.

3. Results and discussion

3.1. Design of the oligodeoxynucleotides

The interaction of α $d(GA)_n$ sequences with $d(GA)_n$ has been explored using a chimeric hairpin substrate. The complementary ends of the hairpin contain β and α d(GA) strands respectively, which can align to form an antiparallel duplex structure. A single T nucleotide was added at the 3′ end to facilitate the synthesis of the substrate using commercially available support and to also provide a potential 3′ protruding nucleotide for intermolecular ligation to a duplex with a single 3′ protruding A nucleotide residue. The chimeric α/β oligodeoxynucleotide can form a hairpin with antiparallel strands in the stem or alternatively a bimolecular duplex with parallel strand orientation (Fig. 1). In addition, multimeric structures with either parallel or antiparallel strand orientation can also be postulated. Oligodeoxynucleotide III was synthesized with an α $(AG)_2$ sequence in the center to determine the type of base pairing between α and β (GA) strands.

3.2. Native gel electrophoresis of oligodeoxynucleotide I

The electrophoretic analysis of oligodeoxynucleotides under non-denaturing conditions is diagnostic for the presence of DNA duplex structure. The mobility of oligodeoxynucleotide I is faster than a single stranded marker of almost identical nucleotide length but the same as a conventional hairpin of eight base pairs in the stem and four residues in the loop (Fig. 1). No evidence of dimeric or multimeric structures for oligodeoxynucleotide I can be observed even at this very high concentration (100 μM strand concentration).

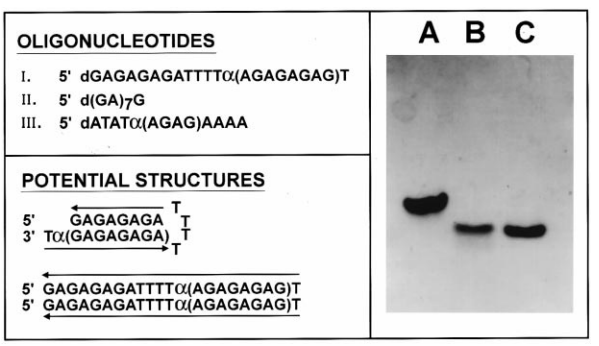

Fig. 1. The left side indicates the sequences of the α and β d(GA) containing oligodeoxynucleotides as well as possible structures oligodeoxynucleotide I might form. The right side is a polyacrylamide gel analysis of oligodeoxynucleotide I under native conditions (see Section 2). Lane A contains a single strand marker (23mer), lane B contains a hairpin marker (TTTTTTTTCCCCAAAAAAAA) and lane C contains oligodeoxynucleotide I.

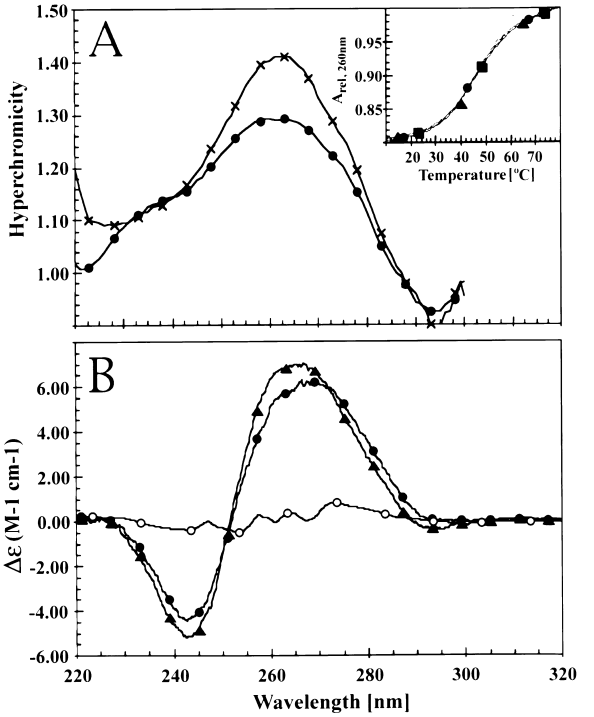

Fig. 2. A: Hyperchromicity profiles of oligodeoxynucleotide I (●) and oligodeoxynucleotide II (×) in 15 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.0. The insert in A shows concentration independent thermal denaturation profiles for oligodeoxynucleotide I in 0.4 M NaCl, 10 mM phosphate, pH 7.0. The strand concentrations were 3 μM (▲), 22 μM (●), and 32 μM (■) respectively. B: A low temperature CD spectrum (5°C) of oligodeoxynucleotide I in 0.4 M NaCl, 10 mM phosphate, pH 7.0, 0.1 mM EDTA (●) and 15 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.0 (▲). A high temperature CD scan (85°C) in 0.4 M NaCl, 10 mM phosphate, pH 7.0, 0.1 mM EDTA is also shown (○).

3.3. Optical properties of the α/β d(GA) hairpin

As previously shown, hyperchromicity profiles of oligodeoxynucleotides are indicative of the nature of the stacking interaction in duplex DNA. For example, parallel and antiparallel DNA duplexes of identical base composition show remarkably different hyperchromicity profiles [25] Thus, a direct comparison of the hyperchromicity profile of the α/β d(GA) hairpin with that of a d(GA) oligodeoxynucleotide of similar sequence but lacking α anomers was diagnostic in the analysis of the α/β d(GA) structure. Analysis of oligodeoxynucleotide I in 15 mM $MgCl_2$ is characteristic of an ordered DNA duplex (Fig. 2A) and is different to that of $d(GA)_7G$ (oligodeoxynucleotide II) under the same conditions. The latter oligodeoxynucleotide has been shown to exist as a parallel stranded homoduplex under these conditions [8]. In addition to the different profile the peak maximum is also slightly shifted. The lower hyperchromicity for the α/β chimera compared to that of the $d(GA)_7G$ duplex is likely due to the five unpaired nucleotides in the intramolecular structure.

The CD spectrum of oligodeoxynucleotide I (5°C) is consistent with the formation of a duplex secondary structure in both 0.4 M NaCl and 15 mM $MgCl_2$ (Fig. 2B). The nearly identical spectra under these two conditions suggest that the structure of the duplex in either 0.4 M NaCl or 15 mM $MgCl_2$ is similar. Heat denaturation (at 85°C) leads to the disappearance of an ordered duplex as is shown in the loss of the peaks. The CD spectra of the α/β d(GA) hairpin are slightly different

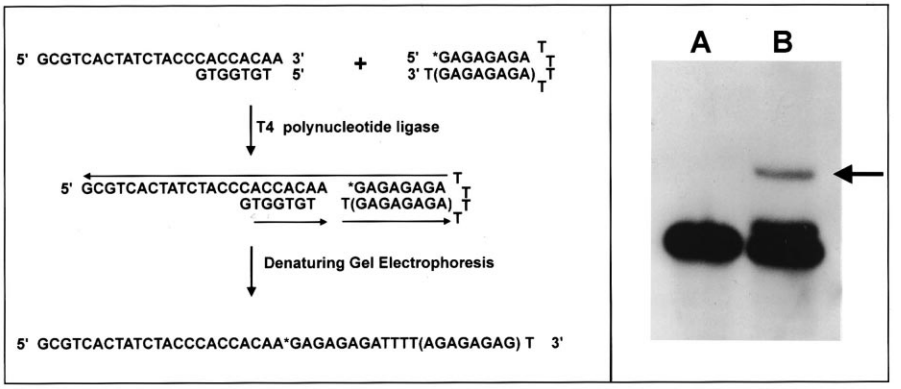

Fig. 3. T_4 polynucleotide ligase promoted joining oligodeoxynucleotide I to an antiparallel duplex. On the left are the duplexes present in the ligase reaction. The brackets indicate the positions of α anomers and the asterisk indicates ^{32}P labeling. On the right are the reactions as analyzed by autoradiography on a denaturing polyacrylamide gel. Lane A has a control reaction with only oligodeoxynucleotide I and single stranded 23mer oligodeoxynucleotide. Lane B has a reaction showing ligation of oligodeoxynucleotide I to the duplex. The arrow indicates the position of the xylene cyanole FF marker, which moves as a 40mer on this gel. An AMP-activated intermediate is seen just above unligated oligodeoxynucleotide I. This intermediate was also noted previously when antiparallel duplexes were ligated to parallel alternating (AT) containing hairpins [24].

from the reported spectra for the parallel duplex of $d(GA)_7G$ in 5 mM $MgCl_2$ [8]. The ratio of the positive to the negative peaks is almost the same in the two duplexes but the antiparallel α/β hairpin lacks the negative ellipticity signal from 290 to 300 nm and the crossovers at 286 and 229 nm reported for the $d(GA)_7G$ parallel duplex.

3.4. UV melting experiments

The UV melting profiles provide for the thermodynamic parameters governing the formation of this novel structure (Table 1). The denaturation profile of oligodeoxynucleotide I was shown to be concentration independent and fully reversible (inset, Fig. 2A). This supports the presence of an intramolecular hairpin structure and is in agreement with the electrophoretic gel which also demonstrated such a structure. The salt dependence of the melting ($d\log[NaCl]/T_m = 13°C$) is characteristic of DNA duplex structure [26]. The UV data were analyzed using a two state melting model (Table 1). The observed T_m for the hairpin formed by oligodeoxynucleotide I is 44.7°C in 0.4 M NaCl and indicates a remarkably stable duplex structure. The comparable enthalpy per nearest neighbor interaction of the novel hairpin at 14 kJ/mol to that of the parallel stranded $d(GA)_n$ duplex [8] at 15–15.7 kJ/mol most likely reflects a similar base stacking in the antiparallel and parallel (GA) duplex structure.

Table 1
Thermodynamic data for 5′-$d(GA)_4(T)_4$ α $d(AG)_4T$-3′

	NaCl[a]	$MgCl_2$[b]
T_m (°C)	44.7 (0.2)	45.2 (0.7)
Enthalpy (kJ/mol)	133.4 (4.9)	131.8 (6.2)
Entropy (kJ/mol/K)	0.420 (0.016)	0.414 (0.019)
$d\log[NaCl]/dT_m$[c]	13°	

The T_m data are the average of 2–4 independent measurements. The values in parentheses indicate standard deviation.
[a]NaCl solutions: 400 mM NaCl, 10 mM phosphate, 0.1 mM EDTA, pH 7.0.
[b]$MgCl_2$ solutions: 15 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.0.
[c]$d\log[NaCl]/dT_m$ was calculated for 0.2–0.5 M NaCl.

3.5. Base pairing in α d(GA)•d(GA) sequences

The optical and electrophoretic data do not provide direct information about the nature of the base pairing in the hairpin duplex formed by oligodeoxynucleotide I. In particular, they cannot distinguish between αG-G and αA-A or αG-A and αA-G base pairing. The ability of T_4 polynucleotide ligase to ligate antiparallel duplexes to unconventional duplexes [24] was used to provide information about the base pairing in this novel structure. If the base pairing in the stem of the antiparallel stranded hairpin is αG-G and αA-A this hairpin will have a 3′-T protruding end. This makes it a potential substrate to ligate to an acceptor duplex containing the appropriate protruding 3′-dA, which is made by combining the 23mer and 7mer oligodeoxynucleotides shown in Fig. 3, left side. The antiparallel hairpin was 5′ ^{32}P end-labeled and added to T_4 polynucleotide ligase reactions with oligodeoxynucleotide (23mer) both in the presence and in the absence of the heptamer second strand.

Analysis of the reactions by denaturing polyacrylamide gel electrophoresis shows that in the presence of the acceptor duplex a new slower moving product with a mobility corresponding to a covalently joined oligodeoxynucleotide I and 23mer (Fig. 3). This new oligodeoxynucleotide is not observed when only 23mer is present in the reaction. This observation

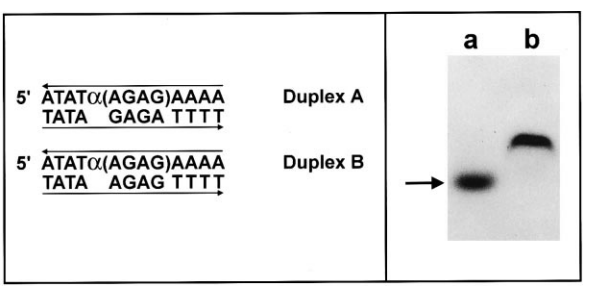

Fig. 4. Left panel: Potential duplexes, A and B, formed between oligodeoxynucleotide III and two oligodeoxynucleotides with $(GA)_2$ cores are indicated. Right panel: A gel shift assay of ^{32}P labeled oligodeoxynucleotide III. Each reaction contains 8 μM oligodeoxynucleotide III. Unlabeled second strands were added to form either duplex A (lane a) or duplex B (lane b). The arrow indicates the mobility of the single stranded oligodeoxynucleotide III.

and the presence of AMP-activated intermediate also suggest the enzyme binds primarily to the antiparallel acceptor duplex and only recognizes the protruding end of the hairpin. Only with αG-G and αA-A base pairing in the stem does the hairpin have a T nucleotide protruding end. Consequently the formation of the ligation product provides evidence for these base pairs.

The base pairing was also probed using oligodeoxynucleotides in which the flanking sequences impose a constraint on duplex formation (Fig. 4). Oligodeoxynucleotide III, containing an α $d(AG)_2$ core, was combined separately with two oligodeoxynucleotides containing (GA) cores in which either αG-A and αA-G (duplex A) or αG-G and αA-A (duplex B) base pairs are facilitated by the surrounding sequence. As evident on the gel, while oligodeoxynucleotide III runs as a single strand in lane a, it forms a duplex in the presence of the complementary strand in lane b. This indicates that only duplex B, which must have αG-G and αA-A base pairs, is stable. This confirms the base pairing suggested by the ligation results.

It is of interest to note that previous studies with αA anomeric nucleotides embedded in conventional DNA duplex structures showed that the αA-A base pairs were considerably more stable than αA-G base pairs [27]. The presence of αA-A and αG-G base pairs in this novel hairpin is also consistent with the dA-dA and dG-dG base pairing suggested in the model of parallel stranded $d(GA)_n$ duplex structures.

4. Conclusion

Previous studies have demonstrated the polymorphism of $d(GA)_n$ sequences, particularly the self association to parallel stranded duplex forms, which limits their use in probing complementary sequences in DNA. We have added to the polymorphic spectrum of d(GA) sequences by the finding that α d(GA) and β d(GA) repeats form an antiparallel duplex structure. The ease of intramolecular duplex formation was exploited by using an oligodeoxynucleotide containing α d(GA) and β d(GA) repeats to show antiparallel stranded hairpin formation by gel electrophoretic and thermal denaturation criteria. The small spectral differences between the antiparallel hairpin and the parallel $d(GA)_7G$ duplex suggests that base stacking and base pairing in these structures are similar. In addition, the enthalpy per residue is very similar in these two antiparallel and parallel helices, which may again indicate structural similarities. Ligation of the hairpin to a duplex as well as a non-denaturing gel duplex assay provide evidence for αG-G and αA-A base pairing in the antiparallel α d(GA)•d(GA) duplex similar to the predicted model for d(GA) sequences [8].

The novel antiparallel αG-G and αA-A base pairs in this study have the potential to be used in the design of either nuclease resistant α/β chimerical antisense oligodeoxynucleotides or the use of α $d(GA)_n$ sequences to probe single stranded (GA) repeats formed in the dismutation of d(GA)•d(TC) duplexes to H form DNA.

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