Relative Stability of Parallel- and Antiparallel-Stranded Duplex DNA[†]

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ABSTRACT: We have recently shown that DNA containing homopolymeric A-T base pairs can form a parallel-stranded intramolecular duplex [van de Sande et al. (1988) *Science* (*Washington, D.C.*) *241*, 551–557]. In the present paper, we demonstrate that parallel-stranded DNA can also be formed in unconstrained bimolecular DNA of appropriate sequence homology. Three deoxyoligonucleotides, a 21-mer [dCCCATATATATTTTTTTTCCC], a ps-15-mer [dTATATATAAAAAAAA], and an aps-15-mer [dAAAAAAAATATATAT], have been synthesized. Annealing of 21-mer and aps-15-mer results in the formation of a conventional antiparallel duplex (aps); however, the combination of 21-mer and ps-15-mer forms a duplex in which the two strands are in a parallel orientation (ps). The parallel-stranded structure was established from the following criteria: (i) The parallel-stranded structure shows a 1:1 stoichiometry of the constituent strands. (ii) Gel electrophoretic mobility of the ps and aps duplexes are similar under native conditions. (iii) Spectroscopic properties of the ps duplex are characteristic for a base-paired structure but are different from the aps structure. (iv) Both duplexes undergo a thermally induced helix to coil transition; however, the melting temperature for the ps duplex is 22 °C lower. (v) The minor groove binding drug Hoechst 33258 shows a reduced affinity for the ps compared to the aps duplex. (vi) The parallel-stranded duplex is not a substrate for DNA *Escherichia coli* polymerase I (Klenow fragment) or AMV reverse transcriptase. Parallel-stranded DNA can exist under normal solution conditions, but competition experiments show it to be thermodynamically less favorable than the conventional antiparallel form.

The antiparallel orientation of complementary strands is a common property of the three major polymorphic families of A-, B-, and Z-DNA (Saenger, 1984; Dickerson, 1988; Zimmerman, 1982). Parallel-stranded duplex structures are possible if bases are appropriately modified or protonated (Westhof & Sundaralingam, 1980; Sarma et al., 1986; Rich et al., 1961). A parallel-stranded duplex structure can also be formed between α-anomeric deoxyoligonucleotides and the complementary natural oligo- (β) deoxynucleotides (Morvan et al., 1987; Thuong et al., 1987; Praseuth et al., 1987). In triple-stranded helices formed between polypurine and polypyrimidine sequences, parallel orientation of two of the strands is associated with Hoogsteen base pairing of one polypyrimidine strand in the major groove of the former double helix (Riley et al., 1966; Lee et al., 1979). The parallel-stranded nature of all these structures is a consequence of unusual chemical or environmental features not present in natural DNA.

Recently, we have demonstrated that stable parallel-stranded DNA containing reverse Watson–Crick A-T base pairs can exist (van de Sande et al., 1988). Intramolecular parallel-stranded hairpin structures (ps) were formed from deoxyoligonucleotides that contained the complementary sequence $(dA)_{10}\cdot(dT)_{10}$ connected by a four-nucleotide loop containing either a 3′–3′ or 5′–5′ phosphodiester linkage (van de Sande et al., 1988). The ps hairpins form duplexes of thermal stability comparable to that of the antiparallel- (aps) stranded hairpins of identical sequence. These findings raise the question whether ps structures could ever be found in unconstrained bimolecular duplex DNA. We now present experimental evidence that conventional A-T deoxyoligonucleotides with appropriate sequence homology can form stable parallel-stranded double-helical structures in the absence of constraining hairpin loops. The design of the duplexes, in which an identical template strand is used for both the ps and aps duplexes, permits the relative stabilities of ps and aps DNA to be compared.

EXPERIMENTAL PROCEDURES

Synthesis and Purification of Deoxyoligonucleotides. Deoxyoligonucleotides were synthesized by using automated phosphoramidite chemistry on a DNA synthesizer (Applied Biosystems, Model 380A). The 5′-tritylated synthesis products were purified by reverse-phase chromatography HPLC on a PRP-1 column as described previously (Germann et al., 1987). Molar extinction coefficients for the deoxyoligonucleotides were obtained from a modified phosphate analysis (Chen et al., 1956).

Spectroscopy. Ultraviolet (UV) absorption spectra and thermal denaturation profiles were recorded with a Varian 2280 spectrophotometer equipped with temperature-regulated cuvette holders. Circular dichroism (CD) measurements were carried out on a Jasco J-500C spectropolarimeter equipped with a temperature control unit.

Thermal Denaturation Analysis. Denaturation was followed at 268 nm. The temperature was increased at 0.5 °C/min; however, changes in the heating or cooling rates (0.1 °C/min–1.5 °C/min) did not affect the thermodynamic parameters. Absorbance readings were collected at 0.5 °C intervals (typically 80 data pairs), corrected for volume expansion, and were fitted to a concerted two-state model by using a Simplex procedure. The absorbances of helix and coil form were allowed to vary linearly with the temperature (Pörschke & Jung, 1982). The melting point is defined as the temperature at which 50% of the duplex is denatured. Enthalpies were assumed to be independent of the temperature within the range considered. Entropies are calculated from $\Delta S^\circ = \Delta H^\circ_{vH}/T_m - R \ln (C_T/4)$, where R is the gas constant, T_m is the melting temperature (Kelvin), and C_T is the total strand concentration of the constituent oligonucleotides (M).

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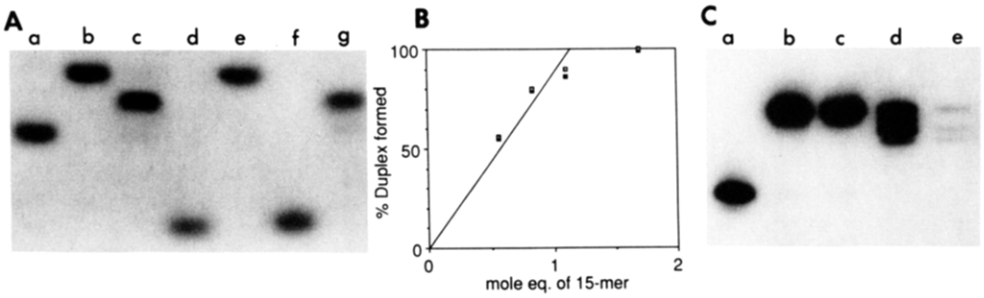

FIGURE 1: (A) Autoradiogram of a 15% nondenaturing polyacrylamide gel (10 mM $MgCl_2$) of ps and aps DNA and their constituent deoxyoligonucleotides. All lanes contained 2 pmol of 5′-end-labeled deoxyoligonucleotides. Unlabeled deoxyoligonucleotides were added at ratios of 15:1. The mixture was heated to 60 °C and then slowly cooled to 4 °C. Samples were applied in running buffer containing 10% sucrose. Lane a, 5′-labeled 21-mer; lane b, 5′-labeled 21-mer and aps-15-mer; lane c, 5′-labeled 21-mer and ps-15-mer; lane d, 5′-labeled aps-15-mer; lane e, 5′-labeled aps-15-mer and 21-mer; lane f, 5′-labeled ps-15-mer; lane g, 5′-labeled ps-15-mer and 21-mer. (B) Determination of the stoichiometry of complex formation of ps and aps DNA. Different amounts (2.0, 3.0, 4.0, and 6.0 pmol) of 5′-end-labeled ps- (⊡) and aps- (■) 15-mers were annealed to 3.6 pmol of the unlabeled 21-mer template strand. The samples were run on a native TBM gel; bands were located by autoradiography, cut out, and quantitated by liquid scintillation counting. The percentage of the counts found in bands corresponding to either the ps or aps complex reflects the fraction of duplexes formed. (C) Autoradiogram of denaturing gel electrophoretic analysis of ps and aps duplex DNA digested with exonuclease I for the time indicated: lane a, 5′-labeled aps-15-mer (marker); lane b, aps duplex components, 0 min; lane c, ps duplex components, 0 min; lane d, aps duplex components, 30 min; lane e, ps duplex components, 30 min.

For the analysis of the concentration dependence of the T_m, the strand concentration was varied from 1.43 to 38 μM. Enthalpies were calculated from the slopes ($R/\Delta H^\circ_{vH}$), and entropies were computed from the intercept [$(\Delta S^\circ - R \ln 4)/\Delta H^\circ_{vH}$] (Aboul-ela et al., 1985).

Enzymatic Reactions. The purified deoxyoligonucleotides were 5′-end-labeled with [γ-^{32}P]ATP and T_4 polynucleotide kinase (Chaconas & van de Sande, 1980). Exonuclease I (5 units) was added to a mixture of 5 pmol of end-labeled 21-mer with a 2-fold excess of either unlabeled ps- or aps-15-mer at 6 °C in TBM buffer. Aliquots were taken and prepared for denaturing polyacrylamide gel electrophoretic analysis by boiling in a solution containing 40% formamide and 0.1 mM ethylenediaminetetraacetic acid (EDTA). *Escherichia coli* polymerase I, large fragment (Klenow), and AMV reverse transcriptase were purchased from Pharmacia and Molecular Genetics Resources, respectively. Two picomoles of 5′-end-labeled aps- or ps-15-mer was annealed to a 2-fold excess of 21-mer at standard Klenow (Maniatis et al., 1982) or AMV reverse transcriptase (Karanthanasis, 1982) reaction conditions in 10 mM $MgCl_2$. Klenow (1 unit) or AMV reverse transcriptase (10 units) was added, and the reactions were carried out for 1 h at 0 or 10 °C as indicated. Aliquots were prepared for denaturing polyacrylamide gel electrophoresis as before.

Gel Electrophoresis. Analysis of deoxyoligonucleotides was carried out on either denaturing 15% polyacrylamide gels run in 8 M urea in TBE buffer (90 mM Tris–borate and 5 mM EDTA, pH 8.3) or 15% polyacrylamide gels run under renaturing conditions in TBM buffer (90 mM Tris–borate, pH 8.3) containing either 10 or 15 mM $MgCl_2$. Kodak X-Omat 5 film was used for autoradiography.

RESULTS AND DISCUSSION

Design of Parallel DNA. The structures of the two DNA duplexes to be studied are

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←                                 ←
5'CCCATATATATTTTTTTTCCC   5'CCCATATATATTTTTTTTCCC
    TATATATAAAAAAAA 5'        5'TATATATAAAAAAAA
                   →          ←
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The common template strand for the potential ps and aps duplex structures is a 21-nucleotide (nt) oligomer that contains three C residues at both the 5′ and 3′ ends in order to facilitate enzymatic studies. Complementary ps- and aps-15-nt oligomers have the identical nucleotide sequence if read in the opposite polarity; i.e., the aps-15-mer has the $(dA)_8$ sequence of the 5′ end while the ps-15-mer has the $(dA)_8$ segment at the 3′ terminus. The duplex part of the DNA structures contains both a homooligomeric dA•dT and an alternating d(A-T) segment. Homooligomeric dA•dT segments have been shown to form parallel duplexes in hairpin structures (van de Sande et al., 1988); however, in an unconstrained system antiparallel duplex formation would be exclusively observed (Cassani et al., 1969). The alternating d(A-T) segment was included to increase the difference in stability between the desired alignment of the complementary strands and any other partially base-paired intra- or intermolecular structures.

Evidence for Parallel-Stranded Structure. The 21-mer, aps-15-mer, and ps-15-mer were 5′-end-labeled with ^{32}P and analyzed by electrophoresis on a polyacrylamide gel under nondenaturing conditions. All molecules showed single bands of expected mobilities; there was no evidence of the formation of any intermolecular structures of the oligomers by themselves (Figure 1A, lanes a, d, and f). Annealing the end-labeled 21-mer template with either the unlabeled aps- or the ps-15-mer resulted, in both cases, in the formation of a single product (Figure 1A, lanes b and c). In neither case did we detect any bands that might be due to concatameric structures. The same results were obtained when the 21-mer was annealed with labeled aps- or ps-15-mers (Figure 1A, lanes e and g). The structure formed between the 21-mer and the ps-15-mer has a faster electrophoretic mobility under native conditions than the conventional antiparallel duplex; this mobility difference was also found for the aps and ps hairpin duplex structures (van de Sande et al., 1988). The stoichiometry of the complexes was further studied in a titration experiment in which increasing amounts of labeled 15-mers were added to the 21-mer. This experiment demonstrates that both structures are formed in a 1:1 stoichiometry of the constituent strands (Figure 1B).

The double-stranded nature of the aps and ps complexes was confirmed by ethidium bromide staining of the bands in native gels (data not shown). Binding of ethidium bromide to the ps duplex in solution elicited a 2-fold higher fluorescence compared to that to the aps duplex; the same difference was found previously (van de Sande et al., 1988). In contrast, the minor groove specific drug Hoechst 33258, which requires the determinants of three to four A-T base pairs (Jorgenson et al., 1988), showed only very weak fluorescence (10%) for the ps duplex but strong fluorescence for the aps duplex. Competition experiments have shown that the basis for this different behavior is the drastically reduced affinity of Hoechst 33258 for ps structures. We note that in the ps duplex the chemical determinants of the A-T base pairs are still present; however, in addition to the different geometry the binding site also contains the methyl groups.

The alignment of the complementary 15-mers with the 21-mer template in either a parallel or antiparallel orientation can be investigated by digestion of the complexes with *E. coli* exonuclease I. In both structures a single-stranded 3′ overhang of three C residues should be a substrate for exonuclease I (Lehman et al., 1964). The ^{32}P-end-labeled 21-mer template annealed to either the aps- or ps-15-mer was treated with *E. coli* exonuclease I, and the reaction products were analyzed by denaturing gel electrophoresis (Figure 1C). For each structure, the 21-mer template strand is degraded to an approximate length of 18 nucleotides (Figure 1C, lanes d and e), consistent with the correct alignment of the 15-mers on the 21-nt template in either a parallel or antiparallel orientation. The ps structure appears to be a better substrate for exonuclease I, probably because the ps duplex is significantly less stable than the aps duplex and degradation of the single-stranded components to monophosphates proceeds readily.

Possible alternative structures in which the alternating A-T and homooligomeric A·T tracts form antiparallel duplexes that are linked by loop structures have been considered. We exclude this possibility for the following reasons. (i) Such a structure would be compact, and thus its electrophoretic mobility should be significantly different from the aps duplex. (ii) Intercalation of eithidium bromide is expected to destabilize this structure, but a stronger binding of the drug is observed. In addition, the presence of the drug does not decrease the thermal stability of the ps duplex. (iii) Alternative dimeric structures formed in an antiparallel orientation contain loop or other single-stranded regions and are therefore substrates for the single-strand-specific nuclease S1. However, the double-stranded part of the ps duplex was found to be resistant to this enzyme (data not shown). In addition, the optical properties described below are analogous to those obtained for the previously characterized parallel-stranded hairpin structures (van de Sande et al., 1988).

Spectroscopic Properties of ps and aps Duplex DNA. Ultraviolet absorption spectral analysis shows a markedly different hypochromic behavior for ps duplex formation compared to that of the conventional aps duplex (Figure 2A). The higher absorbance for the ps duplex is accompanied by a blue shift of 4 nm compared to that of the aps complement. The difference spectrum of the native duplexes (ps − aps) shows a negative peak at 281 nm ($\Delta\epsilon = -0.7$ mM^{-1} cm^{-1}) and a positive peak at 253 nm ($\Delta\epsilon = 1.6$ mM^{-1} cm^{-1}). The crossover point occurs at 267 nm, the same wavelength as the isochromic point. As a control, the difference spectrum of the denatured duplexes at 80 °C is shown to be close to zero, as expected for deoxyoligonucleotides of the same base composition (Figure 2B). This demonstrates that the spectral characteristics of the native ps and aps forms are intrinsic properties of the two duplex structures. The aps duplex shows a high hyperchromicity (>30%) between 240 and 260 nm and sharply decreases at higher wavelengths. In contrast, the ps duplex exhibits a low hyperchromicity below 260 nm and reaches the maximum hyperchromicity (35%) at 270 nm. Since hypochromicity results from a close, stacked alignment of the transition dipoles of the bases, the stacking arrangement in the ps and aps structures must be significantly different. Hyperchromicity profiles calculated from samples in $MgCl_2$ are identical (data not shown).

The hyperchromicity profile (Figure 2C) and the difference spectra of the ps duplex at low temperature are similar to those observed for the ps hairpins (van de Sande et al., 1988). This suggests that base pairing occurs in the parallel-stranded duplex throughout the length of the 15-mer, including the alternating A-T block for which this has not been previously observed. Preliminary results on alternating parallel-stranded A-T hairpins confirm this observation (Germann et al., unpublished results).

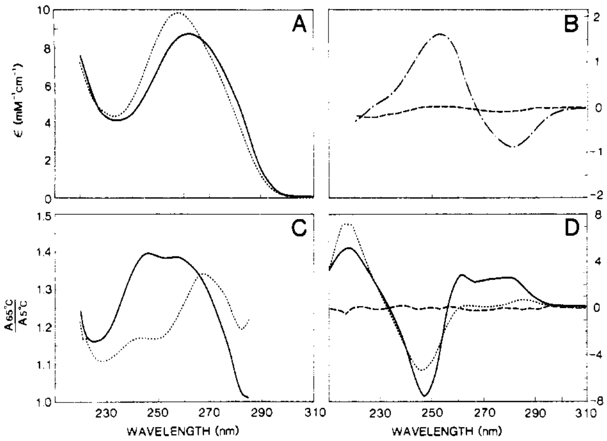

FIGURE 2: (A) Ultraviolet absorption spectra of native ps (···) and aps (—) duplexes in 0.4 M NaCl, 0.1 mM EDTA, and 10 mM phosphate, pH 7.0 at 10 °C. Extinction coefficients are given per residue. (B) Ultraviolet difference spectra (ps − aps) of the native structures at 10 °C (–·–) and the denatured forms (---) at 80 °C in 0.4 M NaCl, 0.1 mM EDTA, and 10 mM phosphate, pH 7.0. (C) Wavelength dependent hyperchromicity of parallel (···) and antiparallel (—) duplexes. Absorbances at 65 °C were divided by the absorbances of the same sample at 5 °C (0.4 M NaCl, 0.1 mM EDTA, and 10 mM phosphate, pH 7.0). (D) Circular dichroism spectra of native ps (···) and aps (—) duplexes in 0.4 M NaCl, 0.1 mM EDTA, and 10 mM phosphate, pH 7.0 at 5 °C, and circular dichroism difference spectrum (ps − asp) at 65 °C (---). Δϵ is given per residue.

The ps and aps duplexes were also analyzed by circular dichroism spectroscopy (Figure 2D). The CD spectra are reminiscent of those obtained for the ps and aps hairpins and again point to structural differences between the two helices. The ps duplex has a slightly higher dichroism than the aps duplex at the positive maximum near 220 nm but lower magnitudes at the longer wavelengths. The zero crossover point at 233 nm is identical for both duplexes, but the second crossover occurs at 259 nm for the ps duplex and at 256 nm for the aps duplex.

The overall similarity of the CD spectra for the ps and aps duplexes is consistent with a right-handed helical structure for the ps duplex. However, we note that the near-ultraviolet CD, in contrast to vacuum CD, is not a reliable indicator for the helical handedness of double-stranded nucleic acids (William et al., 1986). Right-handed helices are characterized by positive CD bands between 180 and 200 nm, followed by a negative band below 180 nm. The recorded vacuum CD spectra for both the aps and ps helices are similar, and both show the characteristic bands expected for right-handed helices (data not shown).

Thermodynamic Properties of ps and aps Duplex DNA. Both the ps and aps duplexes undergo a thermally induced transition from a base-stacked double helix to a denatured coil form (Figure 3A). The ps duplex is considerably less stable than the corresponding aps duplex in 400 mM NaCl; the T_m value of the ps duplex is 21.9 °C lower than that of the aps duplex.

The difference in thermal stability between the ps and aps structures is smaller in the presence of $MgCl_2$ (Table I). This demonstrates a preferential stabilization of the parallel-stranded duplex by divalent ions and presents an incentive to

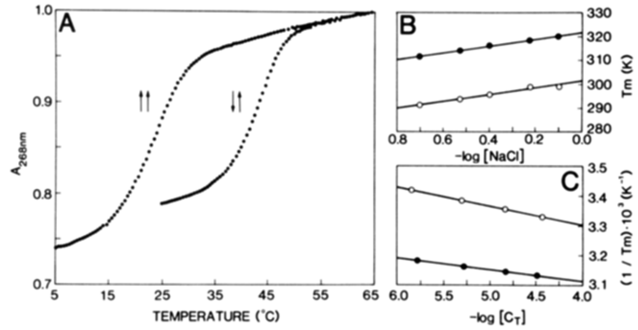

FIGURE 3: (A) Melting profiles of ps (↑↑) and aps (↓↑) duplexes in 0.4 M NaCl, 0.1 mM EDTA, and 10 mM phosphate, pH 7.0. The denaturation was monitored at 268 nm. The total strand concentration (C_T) was 5.05×10^{-6} M. (B) Dependence of the T_m of ps (○) and aps (●) duplexes on the ionic strength (0.1 mM EDTA and 10 mM phosphate, pH 7.0). The strand concentration was 5.05×10^{-6} M. (C) Dependence of the T_m on the total strand concentration of ps (○) and aps (●) duplexes.

Table I: Thermodynamic Data for Helix Formation of ps and aps DNA

	T_m (°C)	ΔH°_{vH} (kJ mol^{-1})	ΔS° (kJ mol^{-1} K^{-1})	dT_m/d log [salt] (deg)
400 mM NaCl[a]				
antiparallel	41.0	−487	−1.42	13.8
parallel	19.1	−309	−0.94	13.9
ΔT_m	21.9			
15 mM MgCl$_2$[b]				
antiparallel	42.7	−480	−1.41	4.6
parallel	24.5	−331	−1.00	3.8
ΔT_m	18.2			

[a] Average melting temperature (T_m) (total strand concentration is 1.43×10^{-6} M) in 10 mM phosphate buffer and 0.1 mM EDTA, pH 7.0. [b] Average melting temperature (strand concentration as above) in 50 mM Tris buffer, pH 7.0. Individual T_m's may vary by ±0.2 °C. ΔH°_{vH} (van't Hoff enthalpy) derived from the concentration dependence of the T_m and from the curve fitting (standard deviation of 9 and 20 kJ mol^{-1} for the ps and aps duplex, respectively). The differences between the average values determined by the two methods were 3% for the ps duplex and 6% for the aps duplex. ΔS° (melting entropy), standard deviation of 0.03 kJ mol^{-1} K^{-1}. The difference in free energy (buffer in footnote b) at 5 °C between the ps and aps structures, calculated from $\Delta G^\circ = \Delta H^\circ_{vH} - T\Delta S^\circ$, is 35 kJ mol^{-1}.

investigate further the effects of cations and ligands on ps duplex stability. The thermally induced helix–coil transitions are monophasic and reversible and can be fitted with high accuracy to a two-state model. The values determined for the melting temperatures (T_m), melting enthalpies ΔH°_{vH}, and entropies ΔS° are wavelength independent and are presented in Table I.

As shown in Figure 3B, both structures show a similar dependence of the T_m on ionic strength. The value for $\partial T_m/\partial$ log [Na$^+$] is constant (13.9°) in the range of 0.2–0.8 M NaCl, indicating a similar degree of counterion association upon formation of the ps and aps duplexes (Manning, 1978). Since the charge spacing in the denatured strands can be assumed to be comparable, it follows that both ps and aps structures have similar charge densities in the native form (Figure 3B).

As expected for bimolecular duplexes, the T_m of both the ps and aps structures is dependent on the duplex concentration (Figure 3C). A variation of 27-fold in strand concentration changes the T_m's of the aps and ps duplexes by 5 and 8.6 °C, respectively. The monophasic nature of the transition and the hyperchromicity observed were independent of the strand concentration. van't Hoff enthalpies (ΔH°_{vH}) calculated from the dependence of the T_m on the strand concentration were in very good agreement to those determined from the analysis of the denaturation profiles (Table I). The ΔH°_{vH} for the denaturation of the ps duplex (309 kJ mol^{-1}) is significantly lower than that observed for the aps DNA (487 kJ mol^{-1}). Calculated enthalpy values for the aps duplex based on nearest-neighbor interactions are in good agreement with the observed value for this duplex (Breslauer et al., 1986). The enthalpy change per mole of nearest-neighbor interaction is 22 kJ mol^{-1} for both the ps hairpins and the ps duplex. This demonstrates an analogous behavior for both the alternating and the homopolymeric segment of the ps duplex.

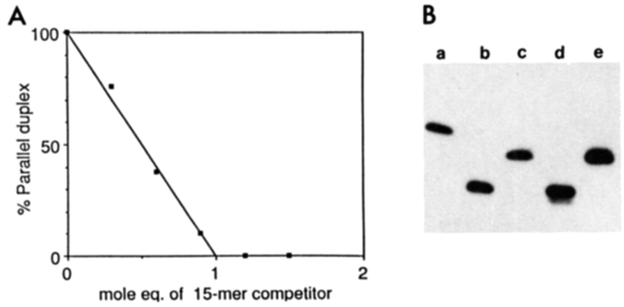

FIGURE 4: (A) Competition experiment. End-labeled 21-mer (6.9 pmol) was mixed with an equal amount of unlabeled ps-15-mer in TBM buffer. Increasing amounts (2.1, 4.1, 6.2, 8.3, and 10.4 pmol) of unlabeled aps-15-mer competitor were added, and the mixture was annealed. Samples were run on a 15% nondenaturing TBM gel (15 mM MgCl$_2$); bands were located by autoradiography and quantitated as described previously. (B) Autoradiogram of denaturing gel electrophoretic analysis of polymerase fill in reactions: lane a, 5′-labeled 21-mer (marker); lane b, ps-15-mer and 21-mer (AMV reverse transcriptase), 10 °C; lane c, aps-15-mer and 21-mer (AMV reverse transcriptase), 10 °C; lane d, ps-15-mer and 21-mer (Klenow), 0 °C; lane e, aps-15-mer and 21-mer (Klenow), 0 °C.

Relative Stability of Parallel-Stranded DNA. The observed differences in gel electrophoretic mobilities between the ps and aps duplexes provide for a simple and direct approach to determine the relative stabilities of the two double-helical structures. Aliquots of unlabeled aps-15-mer were added to the preformed ps duplex containing unlabeled ps-15-mer and the ^{32}P-end-labeled 21-mer template (Figure 4A). Efficient competition occurs; all of the ps duplex was converted to aps duplex on the addition of 1 equiv of the aps-15-mer. Competition experiments were also carried out in the reverse manner, starting with the aps duplex and adding the ps-15-mer competitor. No significant amount (<1%) of ps duplex could be observed, even in the presence of up to a 500-fold excess of the ps strand competitor. From these competition experiments a lower limit for the difference in the free energies for the ps and aps duplexes may be estimated. The aps duplex is favored over the ps duplex by more than 32 kJ mol^{-1}. This agrees closely with our calculated free energy difference of 35 kJ mol^{-1} obtained from the UV melting experiments under the same buffer conditions (Table I).

Substrate Properties of ps and aps Duplex DNA. In both the ps and aps DNA duplexes the 15-mer could act as a primer for enzymatic extension reactions that would occur in the conventional antiparallel manner for the aps duplex, but that would have to take place in a parallel direction for the ps duplex. Reactions were carried out on both duplexes by using either *E. coli* DNA polymerase I large fragment (Klenow fragment) or AMV reverse transcriptase (Figure 4B). Extensions of the aps primer using AMV reverse transcriptase or the Klenow fragment resulted in the production of the expected product of 18 nucleotides in length (Figure 4B, lanes c and e). No extension of the ps primer was observed with either AMV reverse transcriptase or Klenow fragment (Figure 4B, lanes b and d). The partial degradation seen for the ps structure, which is more evident at higher temperatures, can be reproduced with either aps- or ps-15-mers alone in the presence of this Klenow enzyme preparation. In addition, the affinity of Klenow for the parallel-stranded duplex was studied by using nitrocellulose filter binding (Riggs et al., 1970). The Klenow fragment will trap the aps but not the ps duplex on nitrocellulose filters. This shows that the radically different structure of ps DNA allows neither binding nor extension by Klenow or AMV reverse transcriptase.

CONCLUSION

Is parallel-stranded DNA likely to exist in nature? The considerable difference in the stabilities of the ps and aps DNA would argue against a biological role for ps DNA structure. However, as we and others (Ramsing et al., 1988) have demonstrated, this difference in the thermal stability can be modulated by the nature of the counterions and possibly by additional factors as well. For single-stranded DNA segments whose sequences are incompatible with the formation of stable intra- or intermolecular antiparallel structures, parallel duplexes can be formed readily. In addition, the ps structure could potentially play a significant role in RNA–RNA interaction. Our observation that DNA can exist in a ps double-helical form provides yet another example of the remarkable polymorphic nature of DNA. However, the biological significance of parallel-stranded polynucleotides remains to be established.

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