Parallel stranded duplex DNA

Niels B. Ramsing and Thomas M. Jovin*

Department of Molecular Biology, Max Planck Institute for Biophysical Chemistry, 3400 Göttingen, FRG

Received May 31, 1988

ABSTRACT

Three linear 21-nt oligonucleotides (C2, C3, C7) have been synthesized with different sequences of A and T residues. One pairwise combination, (C3, C7), hybridizes to form a conventional antiparallel duplex (aps-C3-C7), whereas the pair C2, C3 forms a duplex (ps-C2-C3) in which the two strands are in a parallel orientation and the A-T base-pairs in a reverse Watson-Crick configuration. The existence of the novel ps helical structure was established from the following criteria: (i) The electrophoretic mobilities of the ps and aps duplexes in native and denaturing polyacrylamide gels are similar. (ii) The ps duplex is not a substrate for T4 DNA ligase. (iii) Salt-dependent thermal transitions are observed for the two duplexes, but the melting temperatures of the ps molecules are 15 °C lower. (iv) The ultraviolet absorption and circular dichroism spectra of the ps duplex are indicative of a base-paired structure, but differ systematically from that of the aps helix. (v) Based on fluorescent measurements, the bis-benzimidazole drug BBI-258 shows a lower affinity for the ps compared to the aps duplex, whereas the opposite preference holds for the intercalator ethidium bromide. We conclude from the present study that parallel stranded DNA is a stable conformation which can arise by interaction between two conventional strands with appropriate sequence homology.

INTRODUCTION

The characteristic features of the three major families of A-, B-, and Z-DNA duplexes are the antiparallel orientation of the sugar-phosphate strands and Watson-Crick base-pairing (1). However, parallel stranded structures can occur (reviewed in Refs. 1 and 2) under the influence of protonation, bulky base substituents, triple helix formation, and chemical modification of the backbone or of the glycosidic linkages. Pattabiraman reported in 1986 a force field calculation for the homopolymeric duplex d(A)₆·d(T)₆ in a parallel right-handed helical conformation with reverse Watson-Crick base-pairing (3). [The dyad symmetry generally associated with normal Watson-Crick base-pairing is incompatible with parallel stranded duplexes (Fig. 6-2 in Ref. 1)]. In order to test this hypothetical structure, we and our colleagues devised a series of hairpin molecules in which 3'-p-3' and 5-p-5' phosphodiester bonds were introduced in the loops consisting of four C nucleotides (molecule psC) or four G nucleotides (molecule psG) in order to force the decanucleotide d(A)₁₀·d(T)₁₀ stems into a parallel orientation (2). Corresponding

reference apsC and apsG hairpins with antiparallel stem regions were synthesized and investigated. It was shown that the ps hairpins are only slightly less thermodynamically stable than the aps molecules and that they exhibit characteristic differences in absorption and CD spectra, enzyme substrate specificities, and drug-binding properties (2). In view of the apparent stability of the ps helix, it appeared likely that ps duplexes could be formed by hybridization of oligonucleotide sequences with a conventional chemical composition but bearing appropriate nucleotide sequences. We demonstrate here that this is the case. (A related study is reported elsewhere: M.W. Germann, B.W. Kalisch, J.H. van de Sande, in preparation).

EXPERIMENTAL SECTION

Oligonucleotide synthesis and characterization

Oligonucleotides C2, C3, and C7 (Fig. 1) were synthesized by conventional phosphoramidite chemistry with an Applied Biosystems Model 381A DNA Synthesizer, and purified by chromatography on a Pharmacia FPLC system with (i) a ProRPC HR5/10 reverse phase FPLC column before detritylation, followed by (ii) a Mono Q HR5/5 anion exchange column after detritylation. Oligonucleotide concentrations are expressed in molar base units unless otherwise indicated. All solutions contained a standard buffer, 10 mM Na-cacodylate, pH 7.2, in addition to the specified salts (NaCl, MgCl₂). The oligonucleotides and their complexes were examined by polyacrylamide gel electrophoresis under two conditions: (i) denaturing: 14 or 20 % polyacrylamide (5 % crosslinking), 7 M urea, 90 mM Tris-borate, 2 mM Na-EDTA, pH 8.0, 50-70 °C, and (ii) native: 12% polyacrylamide (5 % crosslinking), 90 mM Tris-borate, pH 8.0, 2 mM MgCl₂, 20 °C or as otherwise indicated. The reference molecule was a 21-bp duplex of complementary oligonucleotides with 3-nt overlapping ends (a kind gift of S. Diekmann). The gels were stained with (i) fluorescent dyes: 0.6 μM ethidium bromide or 3 μM BBI-258 (our designation for Hoechst-33258, supplied by Serva); and (ii) silver based reactions. For some experiments, the oligonucleotides were end-labeled with [³²P] to a specific activity of 10⁵ cpm/nmol with T4 polynucleotide kinase (Amersham) (4); the labeled molecules are designated with an appended asterisk (*) in the text and figure legends. Autoradiographs from radiolabeled samples and the fluorescence images of gels excited by a transilluminator were measured with a TV camera. The images were digitized and processed by analysis and enhancement techniques. The molar extinction coefficients of the oligonucleotides were determined by phosphate analysis after ashing (5) and with ATP and orthophosphate as standards. The ε₂₆₀ was 8.8 (mmol base)⁻¹ cm⁻¹ (standard deviation 7%) for the three oligonucleotides C2, C3, and C7, in the denatured state (70 °C, standard buffer + 0.1 M NaCl). This value agrees with that reported for denatured poly(dA)-poly(dT) (6). T4 DNA ligase was from New England Biolabs. DNA duplexes were

prepared by mixing the appropriate oligonucleotides in the desired salt solutions, heating to 50-60 °C for 5 min, and cooling slowly to room temperature.

Spectroscopy

Ultraviolet absorption spectra were recorded on a Uvikon 820 spectrophotometer equipped with computer-controlled thermostated cuvette holders and data acquisition. Measurements were made in 4 mm (width) x 1 cm (pathlength) cuvettes at 4 ° intervals for both heating and cooling cycles. Thermal equilibration was achieved in 2 min and a further 4 min were allowed to establish chemical equilibrium. The data sets were corrected and analyzed according to procedures described under Results. The spectra were plotted with the ICEX GRAFkit graphics program for the DEC MicroVax II computer. Circular dichroism (CD) spectra were acquired on a Jobin Yvon Model V Dichrograph in 2 mm (width) x 10 mm (pathlength) cuvettes and with a resolution of 1 nm. Fluorescence measurements were made with an SLM 8000 photon counting spectrofluorimeter. DNA additions were made with a 25 µl Hamilton microsyringe-dispenser unit maintained at a temperature not exceeding that of the thermostated cuvette.

RESULTS AND DISCUSSION

Design of oligonucleotides

Three 21-nt sequences (C2, C3, C7, Figure 1) were selected so as to potentiate the formation of perfect aps (C3, C7) and ps (C2, C3) duplexes while restricting the potentially competitive aps-C2-C3 duplex to a greatly reduced number (a maximum of 13) of possible hydrogen bonded base-pairs.

Fig. 1. Oligonucleotides used in this study and their duplexes. The 5'-ends are designated by the codes C2, C3, C7 and the arrows point to the 3'-ends. The Watson-Crick base-pairs of the antiparallel duplexes are shown by the symbol ● and the reverse Watson-Crick base-pairs of the parallel stranded duplex (ps-C2-C3) by the symbol ○. The aps-C2-C3 duplex is shown with the relative positioning of the strands affording the maximal degree of base-pairing (13 out of 21).

Fig. 2. Electrophoretic analysis of oligonucleotides and the ps-C2-C3 duplex. (a) Autoradiograph of [^{32}P] labeled C2, C3, C7, and the 21-nt reference, run in denaturing 14% polyacrylamide gel (origin at top). Lanes: 1, C2*; 2, C3*; 3, C7*; 4, ref.*. (b) Titration of 106 μM C3* with increasing amounts of C2. The DNAs were mixed in 10 mM Na-cacodylate, pH 7.2, 10 mM NaCl, 2mM MgCl_2 ; heated to 60 $^\circ\text{C}$ for 5 min; allowed to cool slowly to 25 $^\circ\text{C}$; and electrophoresed in a native 12% polyacrylamide gel at 20 $^\circ\text{C}$. The molarity (μM) of C2 in lanes 1-12 was: 0, 28, 57, 68, 79, 91, 102, 114, 125, 136, 170, 227. The positions of the single stranded and duplex molecules are indicated by the letters s and d. The origin is at the top of the right-hand bar. The autoradiographs of the gel were digitized and processed so as to obtain the integrated optical density in arbitrary units (a.u.) for each spot. The results are plotted in (c) as a function of the strand concentration ratio $\text{C2}/\text{C3}^*$. (○) intensity of the C3* migrating as a single stranded species; (●) intensity of the double stranded species.

Characterization of oligonucleotides and their aps and ps duplexes by polyacrylamide gel electrophoresis

The purity of the isolated end-labeled oligonucleotides was established by electrophoresis under denaturing conditions (Figure 2a). No contaminating faster or slower migrating species were detected and the mobilities were virtually identical to that of the 21-nt reference. The generation and stability of duplex structures were examined by gel electrophoresis under carefully controlled conditions with respect to pH, ionic composition, temperature, and added ligands. The formation of the parallel stranded ps-C2-C3 duplex was demonstrated by a titration experiment (Fig. 2b,c) in which [^{32}P]-labeled C3* shifted from a migration position corresponding to the single stranded oligonucleotide to that of the double-stranded duplex upon addition of increasing amounts of unlabeled C2. A quantitative analysis of the autoradiographs

Fig. 3. Electrophoresis and drug binding of oligonucleotides and their duplexes. (a) Native gels with 2 mM MgCl_2 at 21 °C. Lanes with the following DNAs (0.9 μg): 1, C2; 2, C3; 3, C7; 4, ps-C2-C3; 5, aps-C3-C7; 6, C2+C7; 7, ref.. Ethidium bromide staining, fluorescence image. Top and bottom margins correspond to 1:1.7 relative migrations. (b) Native gel as in (a) but with 0.35 μg DNA and run at 13 °C. Silver stained picture, enhanced with digital image analysis. The stabilization of the C2 duplex (lane 1) is attributable to the lower running temperature compared to that in (a) and (c). (c) Native gel as in (a) but stained with BBI-258. Fluorescence image, digitally enhanced, with lower left box 2x relative to the rest of the image. (d) Native gel as in (c) but lanes 1-4 now correspond to C2, C3, ps-C2-C3, and aps-C3-C7, mixed prior to electrophoresis with BBI-258 at a P/D ratio of ~ 25 . Fluorescence image. Origin at the top. (e) Same gel as in (d) but stained after electrophoresis with BBI-258. Fluorescence image.

demonstrated that the complex had a 1:1 stoichiometry with no indication of higher order structures (Fig. 2c). The ps-C2-C3 and the corresponding aps-C3-C7 duplexes had similar electrophoretic mobilities, comparable to that of the 21-bp reference (Fig. 3a). Under conditions maximally favoring duplex formation (high DNA concentration, low temperature, presence of Mg^{2+}), the ps species consistently had a $\sim 5\%$ higher mobility than its aps counterpart, a finding also observed previously with hairpin molecules (2). The individual strands (C2, C3, C7) and a C2 + C7 mixture migrated as single strands or partially stable homoduplexes under the conditions of electrophoresis. Thus, a comparison in Fig. 3 of panel a (fluorescence images from ethidium bromide staining, specific for duplex structures) with panel b (silver staining, indicative of the distribution of total mass) revealed sharp, congruent bands only with the C2-C3 and C3-C7 duplexes. Furthermore, except in the case of the reference molecule (which had overhanging ends), there was no evidence for higher order duplexes in the form of slower

Fig. 4. Ligation of [^{32}P]-labeled oligonucleotides and their ps and aps duplexes. DNAs (200 ng, 30 pmol ends) were incubated with 100 units of T4 DNA ligase in ligation buffer at 16 °C in a total volume of 10 μl . Aliquots (3 μl) were taken at various times of incubation (45 min: lanes 1, 4, 7, 11, 14; 90 min: 2, 5, 8, 12, 15; 180 min: 3, 6, 9, 13, 16) and stopped by addition of 10 mM EDTA and heating to 80 °C. The aliquots were run on a 20 % polyacrylamide denaturing gel. The autoradiographs were digitized. Panels from left to right: C2, C3, C7, ref. (unligated), ps-C2-C3, aps-C3-C7. Origin at top.

moving bands in native gels. The double stranded nature of the aps and ps duplexes was also evident from the fluorescence staining patterns (Fig. 3c) with the A-T specific DNA drug BBI-258 that binds in the minor groove of B-DNA (7). When added to the samples prior to electrophoresis, this drug produced fluorescent bands with an order of intensity aps-C3-C7 > aps-C2-C2 >> ps-C2-C3 (Fig. 3d). The C3 oligonucleotide did not self-associate appreciably under these conditions and the drug fluorescence was seen only in the position of the sample slot (lane 2). The different distribution of base-pair mismatches (A·A, T·T) in the potential partial homoduplexes of C2 and C3 may have accounted for the differential behavior of the two oligonucleotides. The reduced intensity (binding) of BBI-258 with the ps-C2-C3 duplex (lane 3) compared to the aps-C3-C7 species (lane 4) was consistent with observations made previously with hairpin molecules (2) and was confirmed further in solution studies described below. Staining of the whole gel of panel d with BBI-258 after electrophoresis (panel e) demonstrated C3 in the migration position of the single stranded species. (The fluorescence presumably arose from partial renaturation to a homoduplex structure during staining.). A comparison of lanes 1 in panels a, c, and d of Fig. 3 demonstrates that the homoduplex C2-C2 was stabilized significantly by interaction with the drug.

Substrate properties of ps and aps duplexes

The aps-C3*-C7* duplex was a substrate for T4 DNA ligase (Fig. 4, lanes 14-16). However, neither the individual oligonucleotides C2*, C3*, C7* (lanes 1-9) nor the ps-C2*-C3* duplex (lanes 11-13) generated perceptible products. This result is consistent with the

Fig. 5. Ultraviolet absorption spectra of ps-C2-C3 and aps-C3-C7 duplexes. (a) Spectra under native conditions in 2 mM MgCl₂. (●) ps-C2-C3 and (○) aps-C3-C7 at 15 °C, (■, □) the superimposed spectra (ps, aps) at 70 °C. (b) Difference spectra (ps – aps) at 15 °C: in 2 mM MgCl₂ (●) and 0.1 M NaCl (○), and at 70 °C: in 2 mM MgCl₂ (■) and 0.1 M NaCl (□). (c) Hyperchromicity spectra of ps-C2-C3 (●) and aps-C3-C7 (○), expressed as the ratio of absorbances at 70 °C and 15 °C.

observation that ps hairpins can be ligated only with an aps duplex as a partner (2), implying that DNA ligase requires at least one antiparallel molecule in a blunt end ligation reaction. Both the ps-C2-C3 and aps-C3-C7 duplexes were substrates for the *AhaIII* and *DraI* restriction endonucleases having the recognition site ...TTTAAA..., but the cleavage pattern was strand specific, as described elsewhere (N. B. Ramsing & T. M. Jovin, in preparation).

Spectroscopic properties of ps-C2-C3 and aps-C3-C7 duplexes

The ultraviolet absorption spectra of ps-C2-C3 at 15 °C in both NaCl and MgCl₂ solutions showed distinct differences from those of the corresponding control aps-C3-C7 duplex (Fig. 5a). The difference spectra (ps – aps) had characteristic features (Fig. 5b): a positive peak at 250–255 nm, a negative peak at 280–285 nm, and an isosbestic point at 270 nm. The blue shift in the ultraviolet absorption spectrum of the ps helix, reflected in the pattern of hyperchromicity accompanying denaturation (Fig. 5c, discussed further below), was the same as that observed previously for hairpins with d(A)₁₀-d(T)₁₀ stems (2). Inasmuch as the C2, C3, C7 series of

Fig. 6. Circular dichroism spectra of ps-C2-C3 and aps-C3-C7 duplexes at 15 °C. (a) ps-C2-C3 (●) and aps-C3-C7 (○) in 2 mM MgCl₂ + 10 mM NaCl. (b) ps-C2-C3 (●) and aps-C3-C7 (○) in 0.1 M NaCl. (c) Difference spectra (ps – aps), from (a) (—) and (b) (.....). $\Delta\epsilon$ is the difference in the molar extinction coefficient for right and left polarized light. $\Delta\Delta\epsilon$ is the difference between the $\Delta\epsilon$ values for the two structures.

oligonucleotides consist of mixed A-T sequences, this result implies that the spectral features are characteristic of ps duplexes consisting of A-T base-pairs but independent of the precise sequence. That is, they reflect primarily the geometric disposition of the juxtaposed base-pairs in the ps helical arrangement. The ultraviolet spectra under denaturing conditions (70 °C, Fig. 5a) were virtually identical for the three oligonucleotides C2, C3, C7, and provided a means for confirming the molar extinction coefficients derived from chemical analysis (see above).

The ps-C2-C3 and aps-C3-C7 duplexes were also analyzed by circular dichroism (CD) spectroscopy (Fig. 6). The ps duplex had a slightly higher dichroism at the positive maximum near 220 nm but lower magnitudes at the peaks at longer wavelengths, a result also obtained previously with the hairpin molecules (2). Despite some salt dependent differences in the absolute spectra, the difference CD (Fig. 6c) for both MgCl₂ and NaCl solutions were characterized by positive peaks near 215 nm and 250 nm, and negative peaks at 230-240 and 280 nm. The same general features were observed with the hairpins (2), but the latter also displayed a negative difference peak at 255 nm.

Fig. 7. Thermally-resolved ultraviolet absorption spectra of ps and aps helices expressed as the hyperchromicity, the ratio $A_{\lambda}(T)/A_{\lambda}(T_0)$, where T_0 is the initial temperature. Top row, parallel stranded DNAs: ps-C2-C3 in (a) 2 mM MgCl_2 , and (c) 0.1 M NaCl; (e) psC hairpin in 0.1 M NaCl. Bottom row: antiparallel stranded DNAs: aps-C3-C7 in (b) 2 mM MgCl_2 , and (d) 0.1 M NaCl; (f) apsC hairpin in 0.1 M NaCl.

Thermodynamic properties of ps-C2-C3 and aps-C3-C7 duplexes

The ps-C2-C3 and aps-C3-C7 duplexes exhibited a thermally induced hyperchromicity in the ultraviolet absorption spectrum, indicative of cooperative transitions from base-stacked double stranded structures to a melted random coiled state.

We recorded the thermal transitions as a set of absorption spectra obtained at discrete temperature intervals in both the forward and reverse directions. Examples are given in Fig. 7 in the form of hyperchromicity plots, i.e. the absorbances normalized to the values for each wavelength at the initial (low) temperature. This form of representation is very distinctive for different DNA conformations (N.B. Ramsing and T.M. Jovin, in preparation). Each data set, corresponding to a certain duplex and salt composition, was corrected for discrepancies between the sample and reference cuvettes, buffer absorbance, and thermal expansion. Figs. 5c and 7 indicate that the hyperchromicity spectra of ps and aps helices consisting of A·T base-pairs are distinctly different. Whereas the ps-C2-C3 duplex in either MgCl_2 or NaCl solutions and the psC hairpin had a peak near 270 nm, the corresponding aps molecules showed a plateau in the range of 240-270 nm. The peak hyperchromicity (ca. 1.5) of the two stranded ps duplex was greater than that of the psC hairpin, presumably because the spectrum of the latter contains

relatively temperature independent contributions from the C₄ loop (2). All species demonstrated well defined, fully reversible thermal transitions.

The increase in ultraviolet absorbance upon melting and, in the reverse reaction, the decrease upon cooling were used to determine the thermal denaturation profiles of the various duplexes. The data from experiments under various ionic conditions fit well to a concerted, two state model for the heteroduplex helix-coil transition (8),

in which the two complementary strands are represented by C_i and C_j, joined by a central dot in the case of the duplex. If we designate the dissociation constant by K, the fractional dissociation by α, and the total strand concentration as C_t (an equimolar mixture of C_i and C_j), then

$$\alpha^2 + (\alpha - 1)2K/C_t = 0. \quad (2)$$

K_m, the equilibrium constant at the transition midpoint (α = 1/2), is related to the DNA concentration,

$$K_m = C_t / 4. \quad (3)$$

The measured experimental quantity is A_λ, the absorbance at wavelength λ as a function of temperature, given by

$$A_\lambda = [\epsilon^h(1 - \alpha) + (\epsilon_i^c + \epsilon_j^c)\alpha/2]nC_t \quad (4)$$

in which the superscripts h and c of the molar (base units) extinction coefficients refer to the helical and coil forms, respectively, and n is the oligonucleotide chain length. The van't Hoff enthalpy ΔH_{vH} for the concerted, cooperative thermal transition is defined by

$$\Delta H_{vH} = -R\partial \ln K / \partial (1/T) \quad (5)$$

where R is the gas constant and T the absolute temperature. The melting temperature T_m corresponds to the midpoint of duplex dissociation (α = 1/2).

The corrected thermally-resolved absorbance data were analyzed by non-linear regression, using a combination of Eqs. 2-4 and an integrated form of Eq. 5, in order to determine the spectral coefficients in Eq. 4 (by definition wavelength dependent and assumed to be linearly temperature dependent) and the global thermodynamic quantities T_m and ΔH_{vH}. Only the data corresponding to fractional hyperchromicities upon melting > 1.3 were used for the global evaluation of the parameters given in Table 1. However, the individual A_λ vs. T curves were also analyzed in order to establish conformity with the two state model presented above. The entropic contributions were calculated from

$$\Delta G = -RT \ln K = \Delta H - T\Delta S \quad (6)$$

which combined with Eq. 3, yields (see Ref. 8)

$$T_m^{-1} = -(R/\Delta H) \ln C_t + (\Delta S + R \ln 4)/\Delta H. \quad (7)$$

In solutions containing MgCl₂ as well as at high NaCl concentrations, very good fits to the

Fig. 8. Fit to the two state transition model of absorbance profiles for thermal denaturation of the ps-C2-C3 duplex in 0.5 M NaCl. (a) Experimental slices of A_λ vs. T in region of significance (hyperchromicity > 1.3). (b) Simulated surface generated from parameters derived from global analysis (Table 1). (c) Difference between experimental and simulated data. (d) Representative A_{268} vs. T slices for ps-C2-C3 and aps-C3-C7. Points: experimental data; dotted lines: simulated curves.

two state model presented above were achieved, both from individual slices of A_λ vs. T , as well as in global analyses of the data. The representative plots in Fig. 8 demonstrate that the experimental data (panel a) could be reproduced with very high precision (panel b) from the computed linear parameters and the universal values for T_m and ΔH_{vH} , as indicated by the vanishing magnitude of the surface generated as the difference between the experimental and calculated absorbances (panel c), and by superposed A_λ vs. T profiles (panel d). That both T_m and ΔH_{vH} were lower for ps-C2-C3 than for aps-C3-C7 is also evident from Fig. 8d.

In NaCl solutions alone, deviations from a simple behavior were observed in some of the experiments with ps-C2-C3, as evidenced by greater deviations in the computed ΔH_{vH} at different wavelengths and salt concentrations. We attribute this phenomenon to the intervention of competing equilibria involving antiparallel homoduplexes and heteroduplexes. Mg^{2+} appears to exert a differential stabilizing effect on the parallel stranded helix, even at low concentration (2 mM) and in the presence of added NaCl (Fig. 9a).

The final thermodynamic parameters are summarized in Table 1. The values obtained for T_m and ΔH_{vH} were independent of the direction (ascending or descending) of the thermal transition. ΔH_{vH} was also relatively independent of ion concentration (for the ps duplex in $MgCl_2$ and for the aps duplex under all salt conditions tested), and consistently lower (about 25 %) for the ps compared to the aps helix. The enthalpy change per mole of nearest neighbor interaction (in our case, $n - 1 = 20$ interactions/duplex; see Ref. 9), Δh , was 18 kJ and 24 kJ for the ps-C2-C3 and aps-C3-C7 duplexes, respectively, compared to the 22 and 24 kJ

Table 1. Thermodynamic parameters for helix-coil transitions of ps-C2-C3 and aps-C3-C7

Duplex	0.1 M NaCl				2 mM MgCl ₂			
	<i>T_m</i>	ΔH_{vH}	ΔS	ΔG	<i>T_m</i>	ΔH_{vH}	ΔS	ΔG
	°C	kJ·mol ⁻¹	kJ·mol ⁻¹ ·K ⁻¹	kJ·mol ⁻¹	°C	kJ·mol ⁻¹	kJ·mol ⁻¹ ·K ⁻¹	kJ·mol ⁻¹
ps-C2-C3	26	—	—	—	35	350	1.2	6
aps-C3-C7	41	470	1.5	19	47	485	1.5	28

The thermodynamic parameters measured in 0.1 M NaCl, 10 mM Na-cacodylate, pH 7.2, were averages of three measurements. ΔH_{vH} , ΔS , and ΔG are not tabulated for ps-C2-C3 because of lack of reproducibility of the experimental ΔH_{vH} values (200-400 kJ·mol⁻¹), ascribed to deviations from the two state model. The individual estimates of *T_m* differed by less than 1°. Individual estimates of the other parameters fell within a ± 5 % range around the given mean values.

The thermodynamic parameters in 2 mM MgCl₂, 10 mM Na-cacodylate, pH 7.2, were based on a single experiment. Additional experiments in the presence of varying NaCl concentrations are presented in Fig. 9.

The ΔG values are free energies of transition at 25 °C. The *T_m* values are for *C_t* = 2 μM.

calculated for the ps and aps hairpins by assuming no contributions from the loop elements (2; N.B. Ramsing and T.M. Jovin, in preparation).

The *T_m* values of both the ps and aps duplexes showed a classical dependence upon salt concentration (Fig. 9a). At any given NaCl concentration, the melting temperature of ps-C2-C3

Fig. 9. Dependence of melting temperatures for the ps-C2-C3 and aps-C3-C7 duplexes on (a) salt concentration, and (b) *C_t*, the total oligonucleotide strand concentration. ps-C2-C3 (closed symbols), aps-C3-C7 (open symbols). (a) The salt concentration includes the buffer contribution: NaCl (●, ○); 2 mM MgCl₂ + NaCl, (■, □). The solid lines are linear regressions with slopes noted in the text. *T_m* values have been corrected to *C_t* = 2 μM using Eq. 7. (b) The striped lines were calculated from the parameters of Table 1 and Eq. 7.

Fig. 10. Drug binding to ps-C2-C3 and aps-C3-C7 duplexes. (a) Titration of 50 nM BBI-258 in 2 mM MgCl₂ and 10 mM NaCl at 5 °C with ps-C2-C3 (●), aps-C3-C7 (○), C2 (□), and C3 (Δ). (b) Titration of 0.5 μM ethidium bromide in 0.1 M NaCl at 5 °C with the ps and aps duplexes. Same symbols as in (a). (c) Thermal transitions of preformed ethidium-DNA complexes under the conditions of (b) but with ~60 μM DNA. (The signals were scaled to account for slight discrepancies in DNA concentration). Symbols as in (a). For ethidium, $\lambda_{\text{ex}} = 520$ nm, $\lambda_{\text{em}} = 610$ nm; for BBI-258, $\lambda_{\text{ex}} = 355$ nm, $\lambda_{\text{em}} = 465$ nm.

was 15 ° lower than that of aps-C3-C7, as compared to the 10 ° difference observed with the corresponding hairpins (2). The slope $\partial T_m / \partial \log[\text{Na}^+] = 15^\circ$ was constant in the range 0.02 to 1 M NaCl and the same for both ps and aps duplexes (Fig. 9a). This value is comparable to the 18 ° obtained with the hairpins (2), suggesting that the thermodynamic degree of ion dissociation accompanying denaturation is similar for all species. In 2 mM MgCl₂, the T_m values for the ps and aps duplexes were approximately the same as those obtained in 0.2–0.3 M NaCl (Fig. 9a), demonstrating the pronounced stabilization afforded by the divalent cation. In the presence of 2 mM MgCl₂, added NaCl produced only a slight destabilizing effect on the ps-C2-C3 duplex.

The melting temperature increased with the DNA concentration (Fig. 9b), in accordance with Eq. 7. However, the range covered did not permit an independent measure of ΔH_{vH} from the slope (Eq. 7), although the measured points lay close to the line calculated from Table 1, i.e., using the ΔH_{vH} values derived from the complete analyses of the transitions.

We conclude from the above discussion and the ΔG estimates for 25 °C (Table 1), that the ps duplex is stable under physiological conditions, particularly in the presence of Mg²⁺ (and presumably other divalent cations), a fortunate circumstance for enzymatic and other biochemical studies with this form of DNA.

Drug-DNA interactions.

The interactions of the C2, C3, C7 oligonucleotides and the respective ps-C2-C3 and aps-C3-C7 duplexes with various DNA drugs were studied by monitoring the characteristic

aps-C3•C7

ps-C2•C3

enhancement of fluorescence upon binding. Solution (10) and crystallographic (7) studies of one of these compounds, the bis-benzimidazole BBI-258, indicate that the minor groove determinants of 3 to 5 A·T base-pairs, involving major hydrogen bonding contacts with N3 of A and O2 of T, are required for binding. The fluorescence enhancement arising from interaction of BBI-258 with A·T rich B-DNA (11) was also observed upon titration with the individual oligonucleotides C2 and C3 and with the aps-C3·C7 duplex (Fig. 10a). In contrast, the ps-C2·C3 duplex elicited a much lower fluorescence signal. We attribute the effects with the individual strands to the peculiarities of the potential mismatched homoduplexes which apparently are stabilized by the drug, as already noted above (Fig. 3). A similar titration experiment performed with the intercalator ethidium bromide led to a very different result (Fig. 10b). In this case, the ps duplex produced approximately twice the specific increase in fluorescence as the aps duplex. The effects of helix structure on the interactions with ethidium bromide are depicted in Fig. 10c. Preformed drug-DNA complexes were exposed to increasing temperature. The fluorescence signals showed abrupt decreases at the temperatures predicted from the T_m data of Fig. 9a. The relative affinities of the ps and aps duplexes for BBI-258 and ethidium were in qualitative agreement with the properties of the corresponding hairpin molecules (2), and emphasize that the different helical parameters of the alternative DNA conformations dramatically influence the intercalative and groove binding capacities of the nucleic acid, presumably for structural reasons we have discussed elsewhere (2).

Model structures of ps-C2·C3 and aps-C3·C7 duplexes

The stereo pair representations of the ps-C2·C3 and aps-C3·C7 duplexes in Fig. 11 were constructed by procedures used for generating models of the ps- and aps-hairpin molecules with homopolymer d(A)₁₀·d(T)₁₀ stems (2; W. Elhorst, E. v. Kitzing, and T. M. Jovin, in

Fig. 11. Stereo pair representation of the 21-bp ps-C2·C3 and aps-C3·C7 double helices. Idealized models calculated by W. Elhorst according to procedures developed previously for ps and aps hairpins (2; W. Elhorst, E. v. Kitzing, and T. M. Jovin, in preparation). The basic AMBER software package (12) for force field calculations, modified so as to impose helical symmetry constraints (13), was used in conjunction with the Bremersmann optimisation technique (13). In the initial step, the basepairs of both an antiparallel and a parallel stranded poly(dA)·poly(dT) structure were optimised (W. Elhorst, E. v. Kitzing, and T. M. Jovin, in preparation). Because of the 1-nucleotide repeat in this structure the relative positions of constituent nucleotides can be fully described by a simple rotation and translation, allowing the construction of the idealized C2, C3, and C7 sequences, albeit without consideration of the structural distinctions in A·T and T·A junctions. Using these symmetry-element idealized helices, the ps-C2·C3 and aps-C3·C7 duplexes were generated and refined using a harmonic penalty function on the initial coordinates to relax bad contacts. The stereo pair plots were generated with the molecular graphics program SCHAKAL (version 86b) of Dr. E. Keller, University of Freiburg. The thymine base is emphasized by a darker shading.

Fig. 12. Canonical parallel stranded helical structures, depicted schematically. (a) A ps loop embedded in antiparallel (aps) DNA. As in Fig. 1, the region with reverse Watson-Crick base-pairing is designated by open circles bridging the two strands (light and dark) of the helix, whereas the symbols in the aps regions with conventional Watson-Crick base-pairs are solid. The 5'-ends are also marked with closed circles. Two possible DNA sequences compatible with the central ps loop are shown below the structure. The first incorporates a palindromic sequence (designated by a row of dots and a central arrow), while the second envisions the pairing of partially homologous sequences with a central region of reversed complementarity. (Complementary sequences are denoted by apostrophes.) (b) A ps region arising by looping of a single-stranded nucleic acid (DNA, RNA?) with a pair of distant sequences (shown below the structure) with complementarity in the same backbone direction. (c) An isolated ps segment arising by heterologous association of strands with sequences complementary in the same backbone direction (shown below the structure). Each or both of the strands could, for example, be parts of a hairpin loop. Thus, ps helices may help to stabilize tertiary interactions in DNA and RNA.

preparation). The extrapolation in the present study to the more complex ps-C2-C3 and aps-C3-C7 sequences provides approximate structures, the refinements of which will be reported elsewhere. However, the characteristic features of ps-DNA (2,3) can be perceived in Fig. 11: parallel polarity of the two strands, equivalence of the grooves, and reverse Watson-Crick base-pairing. The helical twist is comparable to that of the aps helix, but the helical rise is approximately 7 % greater according to this initial analysis.

Biological implications of the ps helix

This study (and a related investigation, M.W. Germann, B.W. Kalisch, J.H. van de Sande, in preparation) establishes that the properties of DNA duplexes formed by hybridization of conventional strands are virtually identical to those of hairpins constructed with parallel stranded stems (2). We conclude that given the correct sequence relationships (Fig. 12), DNA containing A-T base-pairs can exist as a stable parallel stranded helix (ps-DNA), although a definitive demonstration of this structure awaits crystallographic and high resolution NMR analysis. The relatively small difference in thermodynamic stability of similar ps and aps duplexes (Table 1) confirm the stacking and hydrogen bonding capacities of the reverse Watson-Crick A-T base-pair, proposed as an inherent feature of ps-DNA (3). However, the corresponding properties of reverse G-C base-pairs in runs or interspersed with A-T sequences remain to be established. The possibility that ps-RNA might exist is intriguing in view of the rich structural and functional repertoire of RNA species in general. Three canonical situations in which ps helices could arise by interactions of wholly or partially homologous strands or looping of single stranded nucleic acid of appropriate sequence are shown in Fig. 12. The topological implications of such structures are of great interest, particularly in relation to the potential roles of ps-DNA and ps-RNA in (nonhomologous) recombination, RNA splicing, stabilization of ribosomal RNA, and other cellular processes. In addition, it can be anticipated that specific ligands, particularly proteins, could intervene in order to stabilize and exploit the parallel stranded conformation.

While the oligonucleotides used in this study possessed the selective hybridization properties desired for discrimination between ps and aps heteroduplexes, their tendency to self-associate into homoduplexes was significant. A computer guided design of sequences for which competing heterologous and homologous antiparallel interactions and the formation of concatameric species are minimized has led to the synthesis of a new generation of molecules with more desirable properties, including the potential for extension to greater lengths.

ACKNOWLEDGMENTS

We are indebted to Wim Elhorst for generating the model structures depicted in Fig. 11, to our colleagues for critical reading of the manuscript, and to G. Heim for technical assistance.

*To whom correspondence should be addressed

REFERENCES

1. Saenger, W. (1984) Principles of Nucleic Acid Structure pp. 556, Springer, New York.
2. van de Sande, J.H., Ramsing, N.B., Germann, M.W., Elhorst, W., Kalisch, B.W., v. Kitzing, E., Pon, R.T., Clegg, R.M. and Jovin, T.M. (1988) Science, in press.

3. Pattabiraman, N. (1986) *Biopolymers* 25, 1603-1606.
4. Chaconas, G. and van de Sande, J. H. (1980) *Methods Enzymol.* 65, 75-85.
5. Chen, P.S., Toribara, T.Y. and Warner, H. (1956) *Anal. Chem.* 28, 1756-1758.
6. Riley, M., Maling, B. and Chamberlin, M.J. (1966) *J. Mol. Biol.* 20, 359-389.
7. Pjura, P.E., Grzeskowiak, K. and Dickerson, R.E. (1987) *J. Mol. Biol.* 197, 257-271.
8. Marky, L.A. and Breslauer, K.J. (1987) *Biopolymers* 26, 1601-1620.
9. Breslauer, K.J., Frank, R., Blöcker, H. and Marky, L.A. (1986) *Proc. Natl. Acad. Sci. USA* 83, 3746-3750.
10. Harshman, K.D. and Dervan, P.B. (1985) *Nucleic Acids. Res.* 13, 4825-4835.
11. Stokke, T. and Steen, H.B. (1985) *J. Histochem. Cytochem.* 33, 333-338.
12. Weiner, P.K. and Kollman, P.A. (1981) *J. Comput. Chem.* 2, 287-303.
13. v. Kitzing, E. and Diekmann, S. (1987) *Eur. Biophys. J.* 15, 13-26.