Volume 16 Number 11 1988 Nucleic Acids Research

Unusual duplex formation in purine rich oligodeoxyribonucleotides

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Received January 14, 1988; Revised and Accepted March 17, 1988

ABSTRACT

The purine rich oligodeoxyribonucleotides 1C, d(ATGACGGAATA) and 2C, d(ATGAGCGAATA) alone exhibit highly cooperative melting transitions. Analysis of the concentration dependence of melting, and electrophoretic studies indicate that these oligomers can form an unusual purine rich offset double helix. The unusual duplex is predicted to contain four A·T, two G·C, and four G·A mismatch base pairs as well as a single A base stacked on the 3' end of each chain of the helix. Other possible models for the duplex are unlikely because they are predicted to contain many base pairs of low stability. Changing the central sequence to CGG or GGG should destabilize the duplex and this is observed. The unusual duplex of 2C is more stable than the duplex of 1C indicating that the stability of G·A base pairs is quite sensitive to the surrounding sequence. Addition of 1C and 2C to their complementary pyrimidine strands results in normal duplexes of similar stability. We feel that the unusual duplexes are significantly stabilized by the intrinsic stacking tendency of purine bases.

INTRODUCTION

Base pairs other than the standard Watson-Crick G·C and A·T are involved in many biologically important structures and recently purine-purine mismatched base pairs have attracted considerable interest. G·A base-pairs are known to be involved in stabilizing t-RNA structure and may be important components of the secondary structure of ribosomal RNA[1-3]. G·G and perhaps G·A and G·T base-pairs may contribute to the structure of telomeres at the ends of eukaryotic chromosomes[4-5]. Such mismatched bases also arise in DNA through replication errors and in recombination, and if unrepaired, can lead to mutations[6-8].

G·A base-pairs, produced by mutation, are repaired at quite different efficiencies depending on local sequence and conformation[6-8]. Early work on G·A and G·G base-pairs by Dodson and Wells[9] demonstrated that both

types of mismatches could be incorporated into a $dG_n \cdot dC_n$ duplex. The mismatches lowered the duplex Tm but were quite resistant to single-strand specific nucleases when inserted as single-base mismatches. Longer sequences of G·A or G·G base pairs were more destabilizing and were more sensitive to nucleases. Tinoco and coworkers[10] have conducted a detailed thermodynamic analysis of mismatched base-pairs in an A·T rich sequence of DNA. They also find that mismatches are destabilized with respect to the normal Watson-Crick base-pairs and that mismatches which contain guanine are the most stable. Recent crystallographic and NMR studies have revealed interesting structural features of G·A mismatch base-pairs in DNA sequences[8,11-15]. Quite reasonable duplex conformations, without severe distortions of the DNA backbone, have been found in x-ray analysis of oligomers with G·A pairs in both the anti-anti and anti-syn geometries[14,15]. In the sequence d(CCAAGATTGG), the two central G·A mispairs are highly propeller twisted and this allows formation of an unusual base-pair hydrogen bond between the 2-NH_2 group of guanine in the mispair and the thymine 2-carbonyl group on the adjacent A·T base pair[15]. Orbons et al.[13] have shown that the sequence d(m^5CGm^5CGAGm^5CG), with two potential G·A base-pairs, exists in an environment dependent duplex-hairpin equilibrium but will not adopt the Z-helical conformation even under extreme conditions.

As part of a program to synthesize sequence-specific recognition probes for both medicinal use and physical studies[16-21], we have prepared the oligomers shown in Scheme I and various modified derivatives (to be reported elsewhere):

1	**d(TATTCCGTCAT)**
1C	**d(ATGACGGAATA)**
2	**d(TATTCGCTCAT)**
2C	**d(ATGAGCGAATA)**
3	**d(ATGAGGCAATA)**
4	**d(ATGAGGGAATA)**

SCHEME I

We have investigated the thermodynamics of duplex formation between the oligomer 1 and its complement 1C, and between 2 and its complement 2C. These duplexes are well behaved and have Tm and enthalpy values for the helix to strand transition in the expected range[22]. The most surprising result of these studies, however, is that 1C and 2C alone have cooperative melting transitions with relatively high Tm and enthalpy

values. We propose a new type of purine-rich self-complementary double helix for these molecules which must have four G·A base-pairs in both the 1C and 2C duplexes. It is obvious that unusual duplex formation of this type can interfere with desired sequence specific recognition events.

EXPERIMENTAL

Oligomer Synthesis

Oligodeoxyribonucleotides were synthesized on a 10-μmol scale by means of an Applied Biosystems Model 380B DNA synthesizer that employed long-chain alkylamine-functionalized controlled pore (500Å) glass supports, ß-cyanoethylphosphoramidite reagents (0.1M), and the manufacturer's recommended chemistry cycles using either I_2-lutidine-H_2O or I_2-pyridine as the oxidizing agent, and either dimethylamino-pyridine or, preferably, N-methylimidazole as the catalyst for capping[23]. The 5'-dimethoxytrityl derivative in each case was isolated by HPLC using a linear gradient of acetonitrile (20-50% over 30 min) vs 0.1 M triethylammonium acetate, pH 7, and a PRP-1 (Hamilton) column (7x305 mm) at ca. 60°C and 4 mL/min. The resultant detritylated material was precipitated (3-times) from 70:30 v/v EtOH-H_2O containing 0.33 M NaCl. Yields were in the range of 20-25 mg.

Tm Determinations

Oligomer uv spectral analysis and melting temperature experiments were performed on a Cary 219 spectrophotometer which was interfaced to an Apple IIe microcomputer. Tm experiments were performed in cuvettes which were from a matched set of 1cm pathlength, reduced-volume quartz cells with Teflon stoppers. Up to 5 cells were monitored and thermostated in a 5-position rotatable cell turret. The temperature control was through a Haake PG20 temperature programmer connected to a Haake A81 refrigerated water bath. Temperature changes of the oligomer solution were monitored by a Cary 219 thermistor unit, the thermistor of which inserted into the reference buffer solution through a septum. Nitrogen gas was passed continuously through the sample compartment of the Cary 219 during low temperature measurement to prevent condensation. Tm measurements were initiated near 0°C and the temperature was ramped at 0.5°C per minute until complete Tm curves were obtained for all samples in the experiment. A plot of digitized absorbance values versus temperature was recorded on the Cary 219 chart recorder during the experiment. The absorbance values were

simultaneously collected by the computer, using software which averaged ten absorbance readings for each data point and stored the results on disk for subsequent analysis. Tm values were determined by a nonlinear least-squares fitting program that used sloping baselines in both the low and high temperature regions[10,20,24]. Tm experiments were conducted in PIPES buffer at pH 7.0 containing 10mM PIPES, 1mM EDTA, and Na^+ concentrations up to 1.0M (added NaCl).

Oligomer concentrations were determined using absorbance values at 25°C in PIPES buffer with 0.1M NaCl. Extinction coefficients for the oligomers were calculated from mononucleotide and dinucleotide data using the nearest-neighbor approximation[25]. Some of the purine rich chains had considerable duplex structure at 25°C. To get the concentration of these samples, they were first heated past the transition region and the high temperature baseline was extrapolated back to 25°C. This method and the extinction coefficient calculations apparently work quite well since upon mixing a purine rich and a pyrimidine rich oligomer (eg. 1 and 1C or 2 and 2C), only the duplex structure could be detected in electrophoresis gels at low temperature. The Tm curves under these conditions also exhibited only evidence for monophasic transitions with no detectable amount of residual Tm curves characteristic of the structured purine strands (see Figs. 1 and 2).

Electrophoresis

Polyacrylamide gel electrophoresis experiments were carried out under two buffer conditions in 20% polyacrylamide gels (30:0.8 acrylamide: bisacrylamide) prepared in a Biorad Protean II gel apparatus using 0.75 mm spacers[21,26]. TBM buffer (0.1 M Tris-borate-0.005M Mg^{2+}, pH 8.3) and PIPES buffer with no added NaCl were used in the experiments. The same buffers were used in the electrophoresis reservoirs. Samples were prepared from oligomer stock solutions with gel loading buffer containing 0.05% bromophenol blue plus 8% sucrose. Under TBM buffer conditions the experiment was conducted at 4-6 mA for 20 hours at 5°C. For PIPES buffer conditions, the experiment was conducted at 8 mA for 6 hours at 25°C. In both cases after electrophoresis was stopped, the gel was stained with propidium for approximately 10 minutes and photographed under UV light using a Polaroid MP-4 land camera equipped with a Wratten #9 filter.

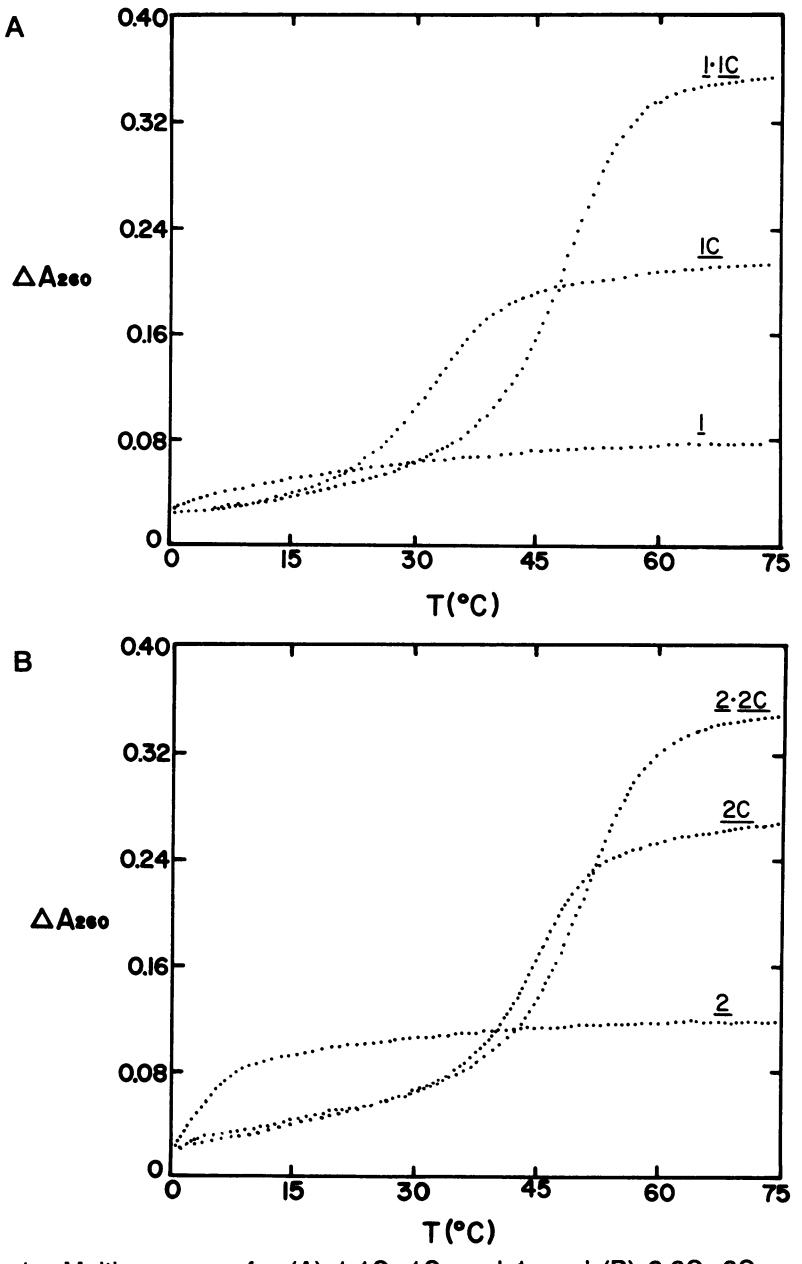

Figure 1. Melting curves for (A) 1·1C, 1C, and 1, and (B) 2·2C, 2C, and 2 in PIPES buffer with 0.40M NaCl. For ease of comparison all curves are normalized to the same starting absorbance and the absorbance change (ΔA_{260}) is plotted versus temperature.

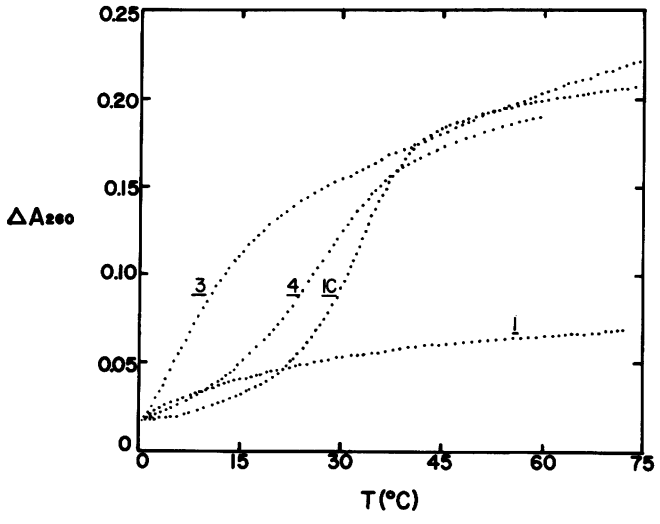

Figure 2. Melting curves for 3, 4, 1C, and 1 in PIPES buffer with 0.40M NaCl.

RESULTS

Sequence Dependence of Melting Behavior

Melting curves for the homogeneous oligomers 1 and 1C as well as the 1:1 heteroduplex of 1 and 1C are shown in Fig. 1A. Similar curves for 2, 2C, and a 1:1 mixture of 2 and 2C are shown in Fig. 1B. The heteroduplex in both cases shows the expected cooperative melting curves. Both pyrimidine rich strands, 1 and 2, alone have some absorbance changes at low temperature, which are characteristic of disruption of weak intrastrand base stacking[27,28], but no melting curves are observed with these oligomers. The most surprising results are obtained on heating solutions of the purine rich strands, 1C and 2C alone. Based on results with other purine rich strands such as poly A[27,28] both 1C and 2C would be expected to show a gradual increase in absorbance, as the temperature is increased, due to purine intrastrand unstacking. Instead, as can be seen in Fig. 1, cooperative melting curves are obtained for both1C and 2C.

Changing the central region of 1C and 2C to either GGC in 3 or GGG in 4 has a dramatic destabilizing effect. In Fig. 2, melting curves for 1 and 1C are compared to curves for 3 and 4. The highest melting oligomer of this series is 1C, 4 is less stable and 3 the least stable of the purine rich strands. No melting curve is obtained for the pyrimidine rich strand, 1.

Concentration Dependence of Melting Behavior

Evaluation of the concentration dependence of melting behavior is a direct method to determine whether oligomers exist as ordered single or double stranded structures at low temperature[10,24,27-29]. For a single stranded structure (such as a hairpin) in equilibrium with a denatured strand the equilibrium position and, therefore, the Tm is not concentration dependent[27,28]. For self-complementary and nonself-complementary duplexes, the equilibrium position and the Tm are concentration dependent. The functional dependence of Tm on concentration is different for homo- and hetero-duplexes. For a self-complementary duplex the Tm depends on concentration as follows:

$$\frac{1}{Tm} = \frac{R}{\Delta H^\circ} \ln \frac{C}{N} + \frac{\Delta S^\circ}{\Delta H^\circ} \qquad (1)$$

where N is one or four for self-complementary or nonself-complementary duplexes respectively, C is the total oligomer single-strand concentration, ΔH° is the enthalpy and ΔS° is the entropy of duplex formation.

Melting curves for 1C, 2C, 3, and 4 alone show a significant concentration dependence and the structured low temperature forms of these chains, therefore, can not be single strands. We feel that triple or higher order strand structures for the unusual conformations of 1C, 2C, 3, and 4 are highly unlikely for several reasons. First, no evidence for biphasic melting is seen under any conditions for these oligomers. With triple helical conformations dissociation of the triple helix to a double helix and a single strand is generally seen at some ionic strengths and this is followed by melting of the duplex to give a biphasic curve[27,28,30]. Second, electrophoretic mobilities for 1C, 2C, 3, and 4 (vidae infra) are similar to those obtained for duplexes of similar size. Third, there is no obvious stable base-pairing possibility for formation of a triple or higher order strand structure with 1C, 2C, 3, or 4[1,27,28,30,31]. Finally, preliminary NMR results are consistent only with formation of a duplex structure[32]. We have, thus, analysed the Tm results of 1C, 2C, and 4 according to a duplex model (eq.1).

The Tm values for 1C, 2C, and 4 are plotted according to eq. (1) for homogeneous chain duplexes (N=1) in Fig. 3A. Tm values for the heteroduplexes, 1·1C and 2·2C, are plotted according to eq. (1) with N=4 in Fig. 3B for comparison. Linear least squares fits to the results in these figures yield enthalpy and entropy values for duplex formation (Table I). All oligomers have favorable enthalpies and unfavorable entropies for

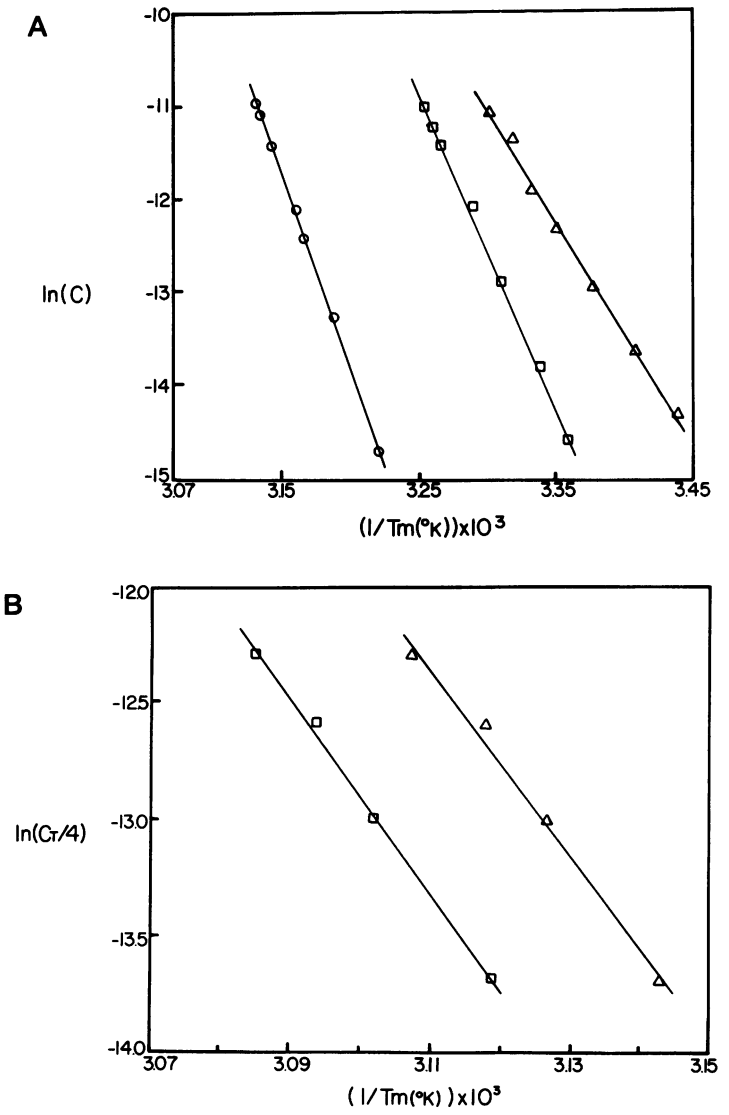

Figure 3. van't Hoff plots for (A) 2C, O; 1C, □; and 4, Δ; and (B) 2C·2, □ and 1C·1, Δ in PIPES buffer with 0.40M NaCl.

duplex formation. Free energies for duplex formation at 25°C are favorable in all cases (Table I). Both of the heteroduplexes and the homoduplex formed from 2C are more stable than the 1C duplex. The duplex from 4 is less stable than 1C and the homoduplex formed from 3 is

Table I. Thermodynamic Constants for Duplex Formation[a]

Oligomer	ΔH°	ΔS°	ΔG°(25°C)
1C	-68.2	-0.200	-8.6
2C	-81.1	-0.232	-12.0
4	-47.0	-0.133	-7.3
1·1C	-78.1	-0.218	-13.1
2·2C	-82.6	-0.230	-14.0

[a]Experiments were performed in PIPES buffer with 0.40M NaCl. Units for ΔH° and ΔG° are kcal/mole and for ΔS° are kcal/mole deg.

the least stable. The duplex from 3 could not be analyzed over a broad enough concentration range to obtain accurate thermodynamic results.

The Effect of Ionic Strength on Melting Behavior

Melting curves for the heteroduplexes 1·1C and 2·2C, and the homoduplexes from 1C, 2C, and 4 were obtained at a range of sodium ion activities and the results are plotted in Fig. 4. The slopes of these plots are directly proportional to the difference in sodium ions associated with the duplex and denatured states of the oligomers, and are inversely proportional to the enthalpy for duplex formation[33,34]. Although the

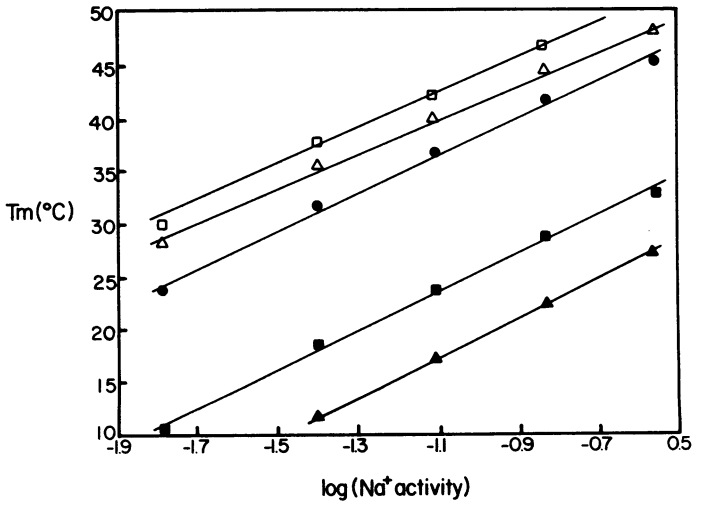

Figure 4. Tm versus log (Na^+ activity) plots for (top to bottom) 2C·2, □ ; 1C·1, Δ; 2C, ●; 1C, ■; and 4, ▲. Homoduplexes are shown with filled symbols and heteroduplexes with open symbols.

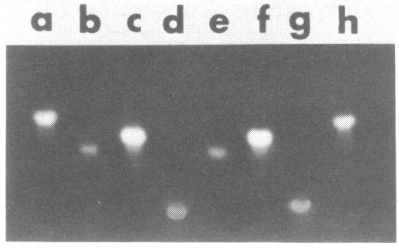

Figure 5. Electrophoresis results in TBM buffer for (a) the Dickerson dodecamer, (b) 1C, (c) 1C·1, (d) 1, (e) 2C, (f) 2C·2, (g) 2, and (h) the Dickerson dodecamer. Because of the weak staining of the oligomers 1 and 2, the bottom of the photograph was exposed for longer than the top to assist in visualization of the fast moving samples. On the gel, their relative intensity is less than for 1C and 2C.

differences in the slopes are close to the experimental error, there is a trend of the slopes in the following order:

$$4 \approx 1C > 2C > 2{\cdot}2C \approx 1{\cdot}1C$$

The Tm for 3 is too low to obtain accurate melting results over a range of salt concentrations. The duplexes 2C, 2·2C, and 1·1C have similar enthalpies of formation, 1C has an intermediate enthalpy, and 4 has the lowest value. At least part of the trend in slopes can, thus, be attributed to differences in enthalpy of duplex formation (Table I) although there may also be subtle differences in ion association for the duplexes which cannot be resolved in the melting experiments.

Electrophoresis of the Oligomers

Electrophoretic results for the oligomers 1, 1C, 1·1C, 2, 2C, and 2·2C are shown in Figure 5 and are compared to results for the Dickerson dodecamer, d(CGCGAATTCGCG). Mobilities, normalized relative to d(CGCGAATTCGCG), are: 1 and 2, 1.35; 1C and 2C, 1.13; 1·1C and 2·2C, 1.08. As expected the Dickerson 12mer has slightly lower mobility than the 1·1C and 2·2C 11mer duplexes. The single strands 1 and 2 have significantly higher mobilities and stain weakly, even at low temperature, with propidium. The strands 1C and 2C show unusual mobilities. Based on their size as single strands, they should migrate close to 1 and 2[21,26] but their mobilities are much closer to mobilities for the duplexes 1·1C and 2·2C than to 1 and 2 as can be seen in Fig. 5.

The same oligomers were also compared in a gel electrophoresis

experiment in PIPES buffer at 25°C. Under these conditions 1, 2, and 1C had the same mobility and the largest mobility of the oligomer series. The oligomer 2C had lower mobility which was slightly greater than the mobility of 1·1C and 2·2C. The Dickerson oligomer again had the lowest mobility but had a small amount of an additional band, which we presume to be the hairpin conformation[35], that had the highest mobility of any band seen on the gel. The mobilities in the PIPES experiment, relative to d(CGCGAATTCGCG), are: 1, 2, and 1C, 1.16; 2C, 1.08; 1·1C and 2·2C, 1.06. Based on the Tm of 1C in low salt, it should be denatured under these conditions and this, no doubt, explains its equivalent mobility to the single-stranded pyrimidine rich oligomers,1 and 2. The other oligomers have significantly higher Tm values and, therefore, migrate largely as duplexes under these conditions.

DISCUSSION

The simplest explanation for all of the observations reported here is that 1·1C and 2·2C form normal B family duplexes, 1 and 2 alone are single stranded species, and 1C and 2C form unusual duplex structures. The most favorable possible structure for the homoduplex of 1C which we have found is shown below:

5' ATGACGGAATA 3'
3' ATAAGGCAGTA 5'

This offset, antiparallel double helix has four A·T and two G·C base pairs of the normal Watson-Crick type, four purine-purine G·A base pairs, and a single unpaired A base at each end of the duplex. It is now well known that G·A base pairs can exist in a B-type helix[8-15]. It is also known that stacked terminal bases, and even terminal phosphate groups, can raise the Tm of oligomer duplexes[20,36]. A hypothetical duplex of 1C which is not off-set:

5' ATGACGGAATA 3'
3' ATAAGGCAGTA 5'

has only two Watson-Crick G·C base pairs, two G·A, four A·A, two T·T, and one G·G mismatched base pairs. Considerable empirical evidence suggests that A·A and T·T are significantly less stable than normal or G·A mismatch base pairs (see 10,37 and references therein). The duplex of 1C offset in the opposite direction:

5' ATGACGGAATA 3'
3' ATAAGGCAGTA 5'

has unfavorable A·A and A·C mismatch base pairs. These latter two structures are, thus, much less likely than the initial duplex shown above.

In the same manner, 2C can form an offset homoduplex which is stabilized by Watson-Crick and G·A base pairs, and terminal A stacking:

5' ATGAGCGAATA 3'
3' ATAAGCGACTA 5'

The nonoffset or reverse offset duplex of 2C also contains base-pairs which should be much less stable than the 2C duplex shown above. Imino proton, nonexchangeable proton, and phosphorous NMR studies in progress[32] indicate that 1C and 2C do form hydrogen bonded duplexes with very similar hydrogen bonding patterns and similar structures. Several unusual chemical shifts are seen in the NMR spectra which suggest that these duplexes are significantly different than the usual B form structure.

The oligomer, 3, has the same composition as 1C and 2C and was designed to test the base pairing schemes shown above for 1C and 2C. The duplex for 3 formed in the same manner as for 1C and 2C:

5' ATGAGGCAATA 3'
3' ATAACGGAGTA 5'

has two A·C in place of two A·G base-pairs and two G·G in place of two G·C base pairs in 1C and 2C and should be much less stable. This is exactly what is observed. Other hydrogen bonding possibilities for the homoduplex of 3 are also unfavorable. The oligomer 4 has three consecutive G bases in the center but should form a more stable duplex than 3. The duplex for 4:

5' ATGAGGGAGTA 3'
3' ATAAGGGAGTA 5'

has two G·G base pairs in place of two G·C base pairs in 1C and 2C and should be of intermediate stability, again as is observed. These results strongly support the offset duplex model for 1C and 2C proposed above.

The enthalpies of formation of the heteroduplexes can be determined from the slopes of the plots in Fig. 3 and can be calculated from the empirical parameters of Breslauer et al[22]. The experimental and calculated values are -78.1 and -78.7 kcal/mole respectively for the 1·1C duplex, and are -82.6 and -80.1 kcal/mole for the 2·2C duplex. These values are in excellent agreement and support the hypothesis that the two heteroduplexes are quite similar and adopt a standard B form structure. The enthalpies for formation of the homoduplexes are -81.1, -68.2, and -47.0 kcal/mole for 2C, 1C, and 4 respectively. There are no standard parameters at present for calculation of enthalpies for mismatched base-

pairs in DNA so these values can not be compared to theory. The decreased stability and enthalpy of duplex formation for 4 is expected due to the replacement of G·C with G·G mismatch base pairs. The significantly enhanced stability of 2C relative to 1C is surprising and can not be explained at this time. These chains have the same composition, base pair types, and very similar sequences. Obviously the stability of duplexes containing G·A base pairs is much more sequence dependent than the stability of duplexes containing the usual Watson-Crick base pairs. Very different values would be calculated for the free energy of formation of G·A base pairs using the 1C and 2C duplexes as basis sets and assuming constant values for the free energy of formation of Watson-Crick base pairs[22]. Tinoco and coworkers[10] have also observed a significant sequence dependence in the effects of G·A mispairs. They find, for example, that the sequence d(CA_3GA_3G)·d(CT_3AT_3G) is more stable at 25°C than the same sequence with the G·A pairs reversed. At higher temperature the two sequences have more similar stability[10].

In summary, gel electrophoretic results and the concentration dependence of melting curves indicate that 1C and 2C form unusual purine rich self-complementary duplexes of significantly different stability. Literature values for the stability of mismatched base pairs and studies of the modified oligomers 3 and 4 strongly support an offset duplex model with four G·A pairs for the unusual duplexes of 1C and 2C. These results suggest that in some purine rich sequences G·A base pairs have significantly enhanced stability and purine rich duplexes can form which are at least partially stabilized by the strong purine base stacking tendency. The enthalpy of G·A and perhaps Watson-Crick base pairs are significantly different between the homo-duplexes of 1C and 2C but the enthalpy of formation of Watson-Crick base pairs in the duplexes 1·1C and 2·2C are quite similar and in agreement with standard values. Unusual duplex formation as a result of G.A pairing could obviously present a problem for analysis of sequence specific processes in DNA interactions. NMR studies are in progress to determine the structures of the purine rich duplexes of 1C and 2C[32].

ACKNOWLEDGMENTS

This work was supported by NSF Grant DMB-8603566. We thank Carol Wilson for assistance in preparation of the manuscript.

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