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The thermal stability of DNA fragments with tandem mismatches at a d(CXYG)·d(CY′X′G) site

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ABSTRACT

Temperature-Gradient Gel Electrophoresis (TGGE) was employed to determine the thermal stabilities of 28 DNA fragments, 373 bp long, with two adjacent mismatched base pairs, and eight DNAs with Watson–Crick base pairs at the same positions. Heteroduplex DNAs containing two adjacent mismatches were formed by melting and reannealing pairs of homologous 373 bp DNA fragments differing by two adjacent base pairs. Product DNAs were separated based on their thermal stability by parallel and perpendicular TGGE. The polyacrylamide gel contained 3.36 M urea and 19.2% formamide to lower the DNA melting temperatures. The order of stability was determined in the sequence context d(CXYG)·d(CY′X′G) where X·X′ and Y·Y′ represent the mismatched or Watson–Crick base pairs. The identity of the mismatched bases and their stacking interactions influence DNA stability. Mobility transition melting temperatures (T_u) of the DNAs with adjacent mismatches were 1.0–3.6°C (±0.2°C) lower than the homoduplex DNA with the d(CCAG)·d(CTGG) sequence. Two adjacent G·A pairs, d(CGAG)·d(CGAG), created a more stable DNA than DNAs with Watson–Crick A·T pairs at the same sites. The d(GA)·d(GA) sequence is estimated to be 0.4 (±30%) kcal/mol more stable in free energy than d(AA)·d(TT) base pairs. This result confirms the unusual stability of the d(GA)·d(GA) sequence previously observed in DNA oligomers. All other DNAs with adjacent mismatched base pairs were less stable than Watson–Crick homoduplex DNAs. Their relative stabilities followed an order expected from previous results on single mismatches. Two homoduplex DNAs with identical nearest neighbor sequences but different next-nearest neighbor sequences had a small but reproducible difference in T_u value. This result indicates that sequence dependent next neighbor stacking interactions influence DNA stability.

INTRODUCTION

Most investigations on the structure and stability of DNA duplexes containing non-Watson–Crick base pairs have focused on isolated defects (1,2). An isolated mismatched base pair or extra base can occur through replication errors, which, if not repaired, lead to mutations (3). Studies have examined DNA oligonucleotides and DNA fragments with isolated mismatched base pairs or an extra base on one strand (a bulge) (4–7). Although the structural and thermodynamic properties of a mismatch or bulge depends on the base(s) involved and their neighboring sequence, DNAs with a mismatch or bulged base are universally less stable than duplexes with Watson–Crick base pairs at the same position.

The physical properties of adjacent mismatched base pairs in DNA are less well characterized. Tandem mismatches are statistically unlikely to arise from random replication errors, but they may occur in folded-back repetitive sequence DNA. The prevalence of repetitive sequence DNA in eucaryotic genomes, and the potential for unusual DNA structures involving mismatches suggest further investigation. Structural studies on adjacent G·A mismatches, d(GA)·d(GA), have been carried out both in the crystal state and in solution (8–11). Unlike single G·A mismatches, which destabilize an otherwise complimentary base paired duplex, adjacent G·A mismatches can stabilize a DNA duplex (11). The conformation and stability of the d(GA)·d(GA) sequence is sensitive to the local context (12). Studies with DNA oligomers show that d(GA)·d(GA) pairs can be more stable than Watson–Crick A·T pairs (13). When flanked by a 5′ pyrimidine and a 3′ purine, the adjacent G·A mismatches adopt an unusual conformation (11,14–16). H-bonding forms between the normally outward facing edges of each purine pair. This conformation allows the adjacent G·A pairs to fit well within a surrounding standard B-form duplex (11). Maskos *et al*. determined that a different pair of adjacent mismatched bases, d(GA)·d(AA), can also form a conformation similar to that of adjacent G·A mismatches (17).

The above observations led us to investigate the thermal stabilities of 28 DNA fragments that contain different adjacent mismatched base pairs. We wished to determine if adjacent mismatches other than d(GA)·d(GA) exhibited unusual stability. Temperature gradient gel electrophoresis (TGGE) was employed to determine the relative stabilities of the DNA fragments using the parallel and perpendicular formats (5,6). In TGGE experiments, a DNA fragment migrates through a polyacrylamide gel with a superimposed thermal gradient. A DNA migrates at a constant mobility until the least stable melting domain unwinds. At this point, the partially melted DNA shows a large decrease in

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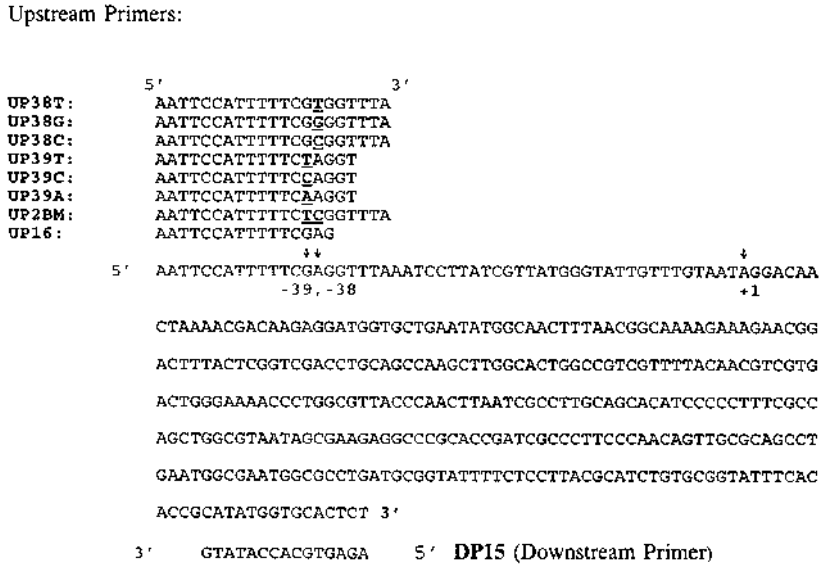

Figure 1. The 373 bp DNA sequence between the *Eco*RI and *Rsa*I sites from pUC8-31 plasmid is shown. Positions –39 and –38 are indicated. The upstream primers, and the downstream primer, DP15, employed in PCR are indicated. Upstream primers created base pair changes at the positions underlined.

mobility. Previous studies have shown that TGGE (5,6,18) and related methods (19) can separate two otherwise identical DNAs differing by a single base pair substitution or defect (mismatch or bulge) in their first melting domain.

MATERIALS AND METHODS

DNA preparation

The plasmid pUC8-31 (5,20) was used as a template in a polymerase chain reaction (PCR) to amplify a variety of 373 bp fragments (Fig. 1). The 373 bp fragments contain the *B.subtilis ctc* promoter sequence and portions of the pUC8 plasmid. Base pair positions are numbered relative to the *ctc* promoter's transcription startpoint. DNA oligonucleotides used as primers for the PCR were from Operon Inc., Alameda, CA. Their sequences and designations are shown in Figure 1. The downstream primer DP15 was end-labeled with ^{32}P for some PCR amplifications using a polynucleotide kinase reaction (21). *Taq* DNA polymerase was obtained from Perkin Elmer Inc. or Promega Inc. Conditions used in the PCR are described in reference 5.

TGGE

TGGE was carried out using an apparatus that resembles a conventional vertical gel electrophoresis unit (5,6,22). Temperature gradients either parallel or perpendicular to the electric field are established with two aluminum heating plates sandwiching the glass plates. Warm and cold fluids from two temperature controlled baths are circulated through the two channels of each heating plate to establish specific temperature gradients. A 6.5% polyacrylamide gel (Protogel, Nat. Diagnostics Inc.) at 37.5:1 acrylamide:bisacrylamide was employed. It contained 3.36 M urea and 19.2% (vol/vol) formamide in 0.5× TBE (0.045 M sodium borate, 0.045 M Tris and 1 mM EDTA, pH 8.1). Formamide was deionized with mixed resin AG501-X8D (BioRad). In parallel TGGE, DNA fragments are loaded into rectangular wells and electrophoresed from a low temperature region (top) to a high temperature region (bottom). The fragments move as duplex molecules until they reach a temperature which causes the least stable melting domain to unwind. The mobility of the partially melted DNA fragments decreases sharply. Based on the size of temperature gradients used, and the ability to distinguish band separation distances of 1.5–2 mm, parallel TGGE can distinguish DNAs with melting temperature differences of ~0.05°C. Ethidium bromide staining is used to visualize the DNA bands.

Perpendicular TGGE was used to obtain the mobility transition curves of the DNAs' first melting domain. In these experiments a temperature gradient is established perpendicular to the direction of mobility (e.g. Fig. 4). The temperature range was selected to focus on the DNAs' first melting domain (6). DNA is loaded into a single long lane across the top of the gel and electrophoresis carried out for 14.5 h at 90 V. The pre-transition increase in DNA mobility reflects temperature dependent changes in gel properties, e.g. pore size and/or viscosity. It is not observed in the absence of a temperature gradient. Subtraction of this baseline corrects for the influence of temperature on the mobility of the duplex. A DNA mobility transition is characterized by a sigmoidal decrease in DNA mobility with increasing temperature (see Fig. 4). The upper slope of the transition represents continued melting beyond the first melting domain. Photographs of the mobility curves were digitized using a digitizer tablet (SummaSketch II). The temperature scale was established by measuring the temperature at several positions across the gel at the end of each run (6). The positions where the needle-like thermocouple probe had been inserted were observed as dark lines in the photographs. Transition curves were smoothed by a locally weighted regression analysis (6). The first melting domain's mobility transition temperature, T_u, was defined as the temperature at the peak of the derivative of the first domain's transition curve.

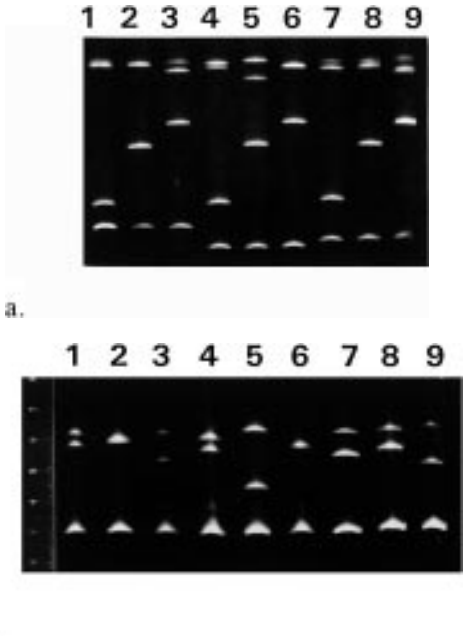

Figure 2. Parallel temperature gradient gel of 373 bp DNAs with tandem mismatches at positions –39 and –38. Samples were run for 17–20 h at 90 V. (**a**) Temperature gradient was from 33.5 to 39°C. From top to bottom in each lane DNA bands are designated by XY·Y′X′, with X and X′ the bases at position –39, and Y and Y′ the bases at position –38: 1) CA·AC, GT·TG, CA·TG, GT·AC. 2) AA·AC and GT·TT, AA·TT, GT·AC. 3) TA·AC, GT·TA, TA·TA, GT·AC. 4) GC·TG, CA·GC, CA·TG, GC·GC. 5) GC·TT, AA·GC, AA·TT, GC·GC. 6) GC·TA and TA·GC, TA·AT, GC·GC. 7) CA·CC, GG·TG, CA·TG, GG·CC. 8) AA·CC, GG·TT, AA·TT, GG·CC. 9) TA·CC, GG·TA, TA·TA, GG·CC. (**b**) Temperature gradient was from 31.5 to 34°C. The top DNA bands containing DNA heteroduplexes in each lane are the same as in (a). The lowest band in each lane contains both homoduplex DNAs, e.g. lane 1) CA·AC, GT·TG, CA·TG and GT·AC.

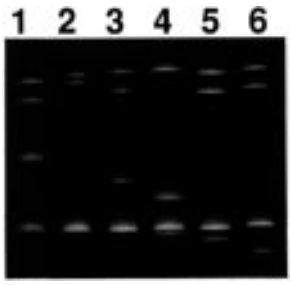

Figure 3. Parallel temperature gradient gel of 373 bp DNAs with base pairs and mispairs at position –39 and –38. Temperature gradient was from 36 to 44°C. Electrophoresis was conducted for 15.5 h at 90 V. From top to bottom in each lane DNA bands contain the following bases at position –39 and –38: 1) TC·TA, TA·GA, TA·TA, TC·GA, 2) TC·TG, CA·GA, TC·GA and CA·TG, 3) TC·TT, AA·GA, AA·TT, TC·GA, 4) TC·TC, GA·GA, TC·GA, GA·TC, 5) TC·AC, GT·GA, TC·GA, GT·AC, 6) TC·CC, GG·GA, TC·GA, GG·CC.

RESULTS

The eight homoduplex DNAs used in this study were generated using PCR as indicated in Figure 1. The DNA fragments differ from each other by one base pair or two adjacent base pairs at sites designated –38 and –39. Pairs of DNAs differing at these positions were melted and reannealed to produce 28 DNAs that contained two adjacent base pair mismatches. All DNA fragments with or without mismatches ran with the same mobility in non-denaturing polyacrylamide gels (not shown). Previous studies showed that the 373 bp homoduplex DNAs have three melting domains (5,20). The first melting domain consists of ~50 bp from the 5′ end of the DNA fragment in Figure 1. It encompasses the positions of the mismatched base pairs.

Figure 2a and b illustrates parallel TGGE experiments of 373 bp DNAs with paired and mismatched bases at position –38 and –39. The identity of the DNA bands are given in the figure caption and were determined using ^{32}P-labeled DNA. One strand of a homoduplex DNA was radioactively labeled using a 5′ ^{32}P-labeled downstream primer in the PCR. After melting a labeled homoduplex DNA and annealing it with a different unlabeled homoduplex DNA, band identities in a temperature gradient gel were made by cutting out the ethidium bromide stained bands and determining which bands were radioactively labeled. Figure 2a used a gradient from 33.5 to 39°C to optimize the separation of base paired and mismatched DNAs in one gel. Figure 2b used a gradient from 31.5 to 34°C to optimize the separation of the DNAs with mismatched bases. Only three bands are observed in each lane of Figure 2b. The lowest band in each lane contains two homoduplex DNAs. The lower denaturing condition used in Figure 2b sacrificed the ability to separate the homoduplex DNAs in order to determine the order of stability for the mismatched DNAs. Other experiments verified the order of stability in the figure legend. Variation in band intensities observed within some lanes were probably due to an unequal amount of the two homoduplexes used to create the four DNAs.

Figure 3 shows a parallel TGGE experiment of 373 bp DNAs with other mismatches at adjacent positions. The identity of the DNAs are given in the figure caption. The two heteroduplex DNAs in lane 1 contain only a single base pair mismatch at position –38. These DNAs allow a comparison to be made between the destabilization caused by one mismatch and two adjacent mismatches.

The most unusual result observed in Figure 3 was the high stability of the d(GpA)·d(GpA) mismatched bases. The DNA with the d(GpA)·d(GpA) sequence at positions –38 and –39 is the second band from the top in lane 4. This band ran further into the gel than the DNAs with d(TpA)·d(TpA) and d(ApA)·d(TpT) at positions –38 and –39. The latter two DNAs are the third band from the top in lanes 1 and 3 respectively. This observation indicates that the d(GpA)·d(GpA) doublet created a more stable DNA than Watson–Crick A·T pairs at the same positions.

Table 1 summarizes the relative stabilities of the 28 DNAs with adjacent mismatches as determined by parallel TGGE. The base identity and the sequence of the mismatches influence the hierarchy of thermal stability. With the exception of d(GpA)·d(GpA), the relative stability of adjacent mismatches parallels the behavior expected from studies of single mismatches. The least destabilizing base to have in adjacent mismatches is G, and the most destabilizing base in adjacent mismatches is C. G·A, G·G and G·T are the least destabilizing mismatches, and C·C and C·T are the most destabilizing mismatches. These results follow the behavior observed for DNAs with single mismatches (4,5,23).

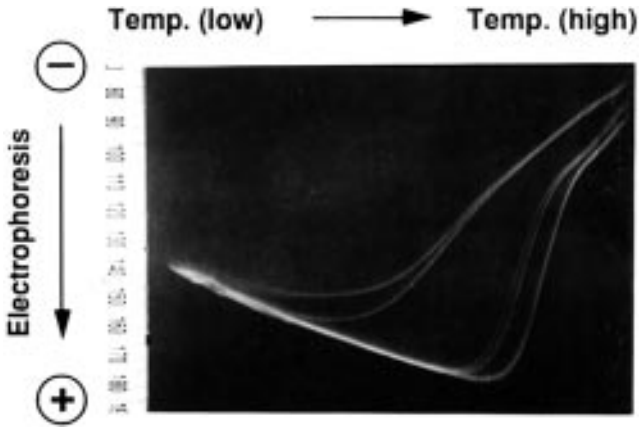

Figure 4. A typical perpendicular temperature gradient gel of 373 bp PCR fragments. The transition curves only show the first melting domain of the DNA fragments. Electrophoresis was conducted for 14.5 h at 90 V. The temperature gradient was 23–44°C from left to right. The sample contained melted and reannealed DNAs with GG·CC and CA·TG at position –39 and –38, and the standard control DNA with GA·TC at the same locus. The transitions from left to right correspond to DNAs with the following bases at –39 and –38: CA·CC, GG·TG, CA·TG, GA·TC, GG·CC. T_u values are 33.9, 34.4, 36.9, 37.1 and 38.0°C.

Table 1. The relative stability of DNAs with two internal adjacent mismatches

GA·GA > **AA·TT** > **TA·TA** > AA·GC > GT·GA > AA·GA > GG·GA > GG·TA > GT·TA > GG·TG > GG·TT = CA·GC = CA·GA≥ GC·TA = TA·GC > GT·TG > AA·AC = GT·TT > GC·TG > CA·CC = CA·AC = TA·AC≥ TC·TG = AA·CC = GC·TT ≥ TA·CC > TC·TT = TC·AC > TC·TC = TC·CC

Notes:
a) The notation WX·YZ represents the 373 bp DNA with the following sequence from positions –40 to –37.
5′—CpWpXpG — 3′
3′—GpZpYpC — 5′
b) The **AA·TT** and **TA·TA** homoduplex DNAs are shown here for comparison.

Perpendicular TGGE experiments were employed to obtain mobility transition curves of the first melting domain of all DNAs. Figure 4 shows typical transition curves of three homoduplex DNAs and two heteroduplex DNAs. The initial increase in DNA mobility with temperature prior to the main transition reflects the effect of temperature on the gel as discussed in Materials and Methods. The two leftmost transition curves correspond to the heteroduplex DNAs with mismatches CA·CC and GG·TG at positions –38 and –39. The broader slope observed with the heteroduplex DNAs was commonly observed. Mobility transition temperatures, T_u, of the DNAs' first melting domain are given in Table 2. In cases where DNAs have the same T_u value, their order is arranged based on results from Table 1, since the resolution of the parallel gradient gel is better than the perpendicular temperature gradient gels.

The T_u values of DNAs with two adjacent mismatches are lower than the T_u value for the wild-type sequence DNA by 1.0–3.6°C ± 0.2°C. The DNA with the d(GA)·d(GA) sequence has the highest T_u value among the DNAs with mismatched base pairs, and is 0.3 and 0.8°C higher than the DNAs with Watson–Crick A·T base pairs. The error in the latter measurements is closer to ±0.1°C since the difference in T_u values are <1°C. The free energy difference between the d(GA)·d(GA) mismatches and the d(AA)·d(TT) sequence can be estimated from the equation, $\delta(\Delta G) = -(\Delta S)\,\delta T_m$ (6,25). ΔS is the entropy change for unwinding the melting domain containing the d(AA)·d(TT) base pairs, and δT_m is the difference in melting temperature between the d(AA)·d(TT) sequence and d(GA)·d(GA) sequence. We assume $\delta T_m \approx \delta T_u = 0.3$°C, and ΔS equals the product of the number of base pairs in the first melting domain, N_1, times the average entropy change/base pair, ΔS^o.

Previous analysis (6) of the 373 bp DNA indicates that $N_1 \approx 52 \pm 4$. Using the average entropy change for melting a base pair of 24.8 cal K^{-1} (mol bp)$^{-1}$ (24), the d(GA)·d(GA) dinucleotide pair is 400 cal/mol (±30%) more stable than d(AA)·d(TT). Using the above approach, free energy differences between the other mismatched DNAs and the DNA with the d(AA)·d(TT) sequence were estimated and are listed in Table 2. It is worth noting that the equation for estimating $\delta(\Delta G)$ assumes T_m differences are small, and similar entropy (or enthalpy) changes for the transitions being compared (25). The former assumption is met by the data. The error in the latter assumption is not certain. Further analysis of the mobility curves is needed to determine if the mismatches alter the size of first melting domain.

The two DNAs produced with the primers UP2BM and UP16 have the top strand sequences d(CTCG) and d(CGAG) respectively from base pair positions –40 to –37 (Fig. 1). These two sequences have identical nearest neighbor interactions, but different next-nearest neighbors at this site. Any difference in the thermal stability between these two DNAs indicates a breakdown of the nearest neighbor model of DNA stability. The lower two bands in lane 4 of Figure 3 are these two DNAs. The DNA with the d(CGAG) sequence ran consistently farther into the parallel temperature gradient gel than the DNA with the d(CTCG) sequence. Perpendicular temperature gradient measurements also showed a slight but reproducible difference (~0.1°C) in the T_u values of their mobility transitions. The free energy difference in the stability of these two DNAs is estimated to be $\delta(\Delta G) \approx$ 130 cal/mol from an analysis analogous to that given above. Werntges *et al.* (26) have also observed melting curve deviations from the expectation of the nearest neighbor model in DNA oligomers.

To compare the effect of nearest neighbor and next nearest-neighbor interactions on mismatched base pairs, two heteroduplex DNAs were produced by melting and reannealing the DNAs containing d(CGCG) and d(CTAG) sequences from positions –40 to –37. Both sequences have a 2-fold symmetry at this locus. The resultant heteroduplex sequences are d(CGCG)·d(CTAG) and d(CTAG)·d(CGCG) and thus have the same nearest neighbor interactions, but different next-nearest neighbor interactions. In contrast to the phenomena observed for Watson–Crick base pairs, there was no observable difference in the thermal stability between these two DNAs, (Fig. 2). The top band in lane 6 of Figure 2a and b contain these two DNAs. Both DNAs migrated to the same position in the TGGE gel indicating the same thermal stability.

Table 2. T_u values of first melting domain of 373 bp DNAs, and estimates of relative free energies

GC·GC:	38.8	GG·CC:	38.0	GT·AC:	37.4	GA·TC:	37.1
TC·GA:	37.0	CA·TG:	36.9	AA·TT:	35.8	TA·TA:	35.3
GA·GA:	36.1 (–0.4)	AA·GC:	34.7 (1.4)	GT·GA:	34.5 (1.7)	AA·GA:	34.5 (1.7)
GG·GA:	34.5 (1.7)	GG·TA:	34.4 (1.8)	GT·TA:	34.4 (1.8)	GG·TG:	34.4 (1.8)
GG·TT:	34.3 (1.9)	CA·GC:	34.3 (1.9)	CA·GA:	34.3 (1.9)	GC·TA:	34.3 (1.9)
TA·GC:	34.3 (1.9)	GT·TG:	34.2 (2.0)	AA·AC:	34.0 (2.3)	GT·TT:	34.0 (2.3)
GC·TG:	34.0 (2.3)	CA·CC:	33.9 (2.5)	CA·AC:	33.9 (2.5)	TA·AC:	33.9 (2.5)
TC·TG:	33.9 (2.5)	AA·CC:	33.9 (2.5)	GC·TT:	33.9 (2.5)	TA·CC:	33.9 (2.5)
TC·TT:	33.7 (2.7)	TC·AC:	33.7 (2.7)	TC·TC:	33.5 (3.0)	TC·CC:	33.5 (3.0)

Notes:
a) T_u is defined as the temperature at the peak of the derivative curve calculated from the smoothed DNA mobility transition profile. Estimated precision in T_u relative to the standard DNA fragment is ±0.2°C. For two DNAs run on the same gel with $\Delta T_u < 1°C$, the precision is within ±0.1°C. Each experiment contained the pUC8-31 DNA fragment as an internal standard. The mean T_u of this DNA was 37.1°C (±0.4°C) based on 22 repeated experiments.
b) The top two rows show the results of homoduplex DNAs. The remaining rows display the results for heteroduplex DNAs.
c) The relative free energy, δ(ΔG), of DNAs with tandem mismatches was estimated with respect to the DNA with the AA·TT base pairs as described in the text. It is given within parentheses in units of kcal/mol ±30%.

DISCUSSION

Ebel *et al.* (13) showed that tandem G·A mismatches in a d(CGAG)·d(CGAG) sequence is more stable than the d(CTAG)·d(CTAG) sequence in a DNA oligomer. Our results verify this observation in a long DNA fragment and show further that the d(CGAG)·d(CGAG) sequence is more stable than the d(CAAG)·d(CTTG) sequence. The unusual stability of tandem G·A pairs requires a 5′Py–G–A–Pu3′ sequence and involves GNH_2–AN7 and GN3–ANH_2 (edge to edge) hydrogen bonds and cross-strand stacking of the adjacent guanine and adenine bases (11–16,27).

The possibility that other tandem mismatches may create unusually stable duplexes was suggested by the studies of Maskos *et al.* (17) on the oligonucleotide $d(GCGAATAAGCG)_2$. This oligomer forms a duplex containing two copies of the mismatched sequences d(CGAA)·d(TAAG) and two 3′ unpaired guanosines. NMR analysis indicates the adjacent G·A and A·A mismatches have an overall duplex structure similar to the adjacent G·A mismatches. Although the d(GA)·d(AA) sequence was among the more stable tandem mismatches in the DNAs we examined, it is not more stable than a pair of Watson–Crick A·T base pairs. Table 2 shows that none of the other adjacent mismatches examined had a stability greater than Watson–Crick A·T base pairs. Other sequence contexts may give different results.

The relative stability of the tandem mismatches shows a trend with regard to purine/pyrimidine content. Sequences with more purines tend to be more stable than mismatches with pyrimidines. The six most stable tandem mismatches have at least one of the most stable single mismatches in DNA, G·T, G·G or G·A, and the six least stable tandem mismatches have at least one of the least stable single mismatches, T·C or C·C (4,5). The range in stability among the tandem mismatches is ~3.4 kcal/mol (Table 2). The stability of 11 tandem mismatches in a r(CXYG)· r(CX′Y′G) context have been measured in RNA oligomers (28). Unfortunately the tandem RNA mismatches examined and the solvent employed differ from the current work making a comparison difficult.

One of the unexpected outcomes of this study was evidence for sequence-dependent next neighbor base pair stacking interactions. The DNAs generated with the primers UP2BM and UP16 (Fig. 1) have identical nearest neighbor base pair sequences yet show a slight difference in DNA stability. Since unwinding the first melting domain of these DNAs does not involve strand dissociation, their difference in stability cannot be explained by differences in strand dissociation parameters. The estimated free energy difference between the two homoduplex DNAs with identical nearest neighbor base pairs, 130 cal/mol, may be viewed as a limit to the accuracy of evaluated nearest neighbor stacking interactions. Deviations between experimental DNA melting curves and theoretical predictions using nearest neighbor stacking parameters may be due to this second order effect (24). Such deviations may not be evident with large melting domains where averaging of longer than nearest neighbor effects minimizes this effect. It may be more apparent for shorter melting domains. The second neighbor effect was not observed when two different DNAs were examined with two adjacent mismatches with identical nearest neighbor sequences. For this situation, the nearest neighbor stacking interaction model appears to be valid.

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REFERENCES

1 Kennard,O. and Hunter,W.N. (1990) *Q. Rev. Biophys.* **23**, 327–379.
2 Patel,D.J., Shapiro,L. and Hare,D. (1987) In Lilly,D. and Eckstein,E. (eds) *Nucleic Acids and Molecular Biology.* Springer-Verlag, Berlin, Vol. 1, pp. 70–84.
3 Lewin,B. (1994) *Genes V.* Oxford University Press Inc., New York, NY.
4 Aboul-ela,F., Koh,D., Tinoco,I.Jr, and Martin,F.H. (1985) *Nucleic Acids Res.*, **13**, 4811–4824.
5 Ke,S-H. and Wartell,R.M. (1993) *Nucleic Acids Res.* **21**, 5137–5143.
6 Ke,S-H. and Wartell,R.M. (1995) *Biochemistry* **34**, 4593–4600.
7 LeBlanc,D.A. and Morden,K.M. (1991) *Biochemistry* **30**, 4042–4047.

8 Kan,L-S., Chandrasegaran,S., Pulford,S.M. and Miller,P.S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4263–4265.
9 Prive,G.G., Heinemann,U., Chandrasegaran,S., Kan,L-S. , Kopka,M.L., and Dickerson,R. (1987) *Science*, **238**, 498–504.
10 Nikonowicz, E. P., Meadows,R.P., Fagan, P. and Gorenstein, D.G. (1991) *Biochemistry* **30**, 1323–1334.
11 Li,Y., Zon,G. and Wilson,W.D. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 26–30.
12 Li,Y., Zon,G. and Wilson,W.D. (1991) *Biochemistry* **30**, 7566–7571.
13 Ebel,S., Lane,A.N. and Brown,T. (1992) *Biochemistry* **31**, 12083–12086.
14 Lane,A., Martin,S.R., Ebel,S. and Brown,T. (1992) *Biochemistry* **31**, 12087–12095.
15 Chou,S.-H., Cheng,J-W. and Reid,B.R. (1992) *J. Mol. Biol.* **228**, 138–155.
16 Cheng,J.-W., Chou,S-H. and Reid,B.R. (1992) *J. Mol. Biol.* **228**, 1037–1041.
17 Maskos,K., Gunn,B.M., LeBlanc,D.A. and Morden,K.M. (1993) *Biochemistry* **32**, 3583–3595.
18 Riesner,D., Henco,K., and Steger,G. (1991) In Chrambach,A., Dunn,M.J. and Radola,B.J. (eds) *Advances in Electrophoresis.* VCH Pub., New York, Vol. 4, pp.171–250.
19 Lerman,L.S., Fischer,S.G., Hurley,I., Silverstein,K. and Lumelsky,N. (1984) *Annu. Rev. Biophys. Bioeng.* **13**, 399–423.
20 Tatti,K.M. and Moran,C.P.Jr (1985) *Nature* **314**, 190–192.
21 Sambrook,J., Fritsch, E.F. and Maniatis,T., (1989) *Molecular Cloning: A Laboratory Manual.* 2nd edition, Cold Spring Harbor Lab. Press, Cold Spring Harbor, NY.
22 Wartell,R.M., Hosseini,S.H. and Moran,C.P.Jr (1990) *Nucleic Acids Res.* **18**, 2699– 2750.
23 Gaffney,B.L. and Jones,R.A. (1989) *Biochemistry* **26**, 5881–5889.
24 Delcourt, S.G. and Blake, R.D. (1991) *J. Biol. Chem.* **266**, 15160–15169.
25 Cantor,C.R. and Schimmel, P.R. (1980) *Biophysical Chemistry, Vol. III.* W.H. Freeman & Co., San Francisco, CA.
26 Werntges,H., Steger,G., Riesner,D. and Fritz,H–J. (1986) *Nucleic Acids Res.* **14**, 3773– 3790.
27 Greene,K.L., Jones,R.L., Li,Y., Robinson,H., Wang,A.H–J., Zon,G. and Wilson,W.D. (1994) *Biochemistry* **33**, 1053–1058.
28 Wu,M., McDowell,J.A., and Turner, D.H. (1995) *Biochemistry* **34**, 3204–3211.