Nucleic Acids Research, 1993, Vol. 21, No. 22 5137–5143

Influence of nearest neighbor sequence on the stability of base pair mismatches in long DNA: determination by temperature-gradient gel electrophoresis

Song-Hua Ke and Roger M.Wartell*
School of Biology, Georgia Institute of Technology, Atlanta, GA 30332, USA

Received July 27, 1993; Revised and Accepted October 4, 1993

ABSTRACT

Temperature-gradient gel electrophoresis (TGGE) was employed to determine the thermal stabilities of 48 DNA fragments that differ by single base pair mismatches. The approach provides a rapid way for studying how specific base mismatches effect the stability of a long DNA fragment. Homologous 373 bp DNA fragments differing by single base pair substitutions in their first melting domain were employed. Heteroduplexes were formed by melting and reannealing pairs of DNAs, one of which was ^{32}P-labeled on its 5′-end. Product DNAs were separated based on their thermal stability by parallel and perpendicular temperature-gradient gel electrophoresis. The order of stability was determined for all common base pairs and mismatched bases in four different nearest neighbor environments; d(GXT)·d(AYC), d(GXG)·d(CYC), d(CXA)·d(TYG), and d(TXT)·d(AYA) with X,Y = A,T,C, or G. DNA fragments containing a single mismatch were destabilized by 1 to 5°C with respect to homologous DNAs with complete Watson–Crick base pairing. Both the bases at the mismatch site and neighboring stacking interactions influence the destabilization caused by a mismatch. G·T, G·G and G·A mismatches were always among the most stable mismatches for all nearest neighbor environments examined. Purine·purine mismatches were generally more stable than pyrimidine·pyrimidine mispairs. Our results are in very good agreement with data where available from solution studies of short DNA oligomers.

INTRODUCTION

Non Watson–Crick or 'mismatched' base pairs occur during DNA replication, genetic recombination and from chemical reactions in cells (1,2,3). The frequency at which a base pair becomes a mutation depends on the frequency of mismatch formation, and the efficiency of mismatch removal by proofreading or repair. Statistical analysis of extant genes and pseudogene sequences indicate that spontaneous mutations do not occur with equal rates for all base pairs (4). The type of base pair substitution and the local sequence environment influence mutation rates. How neighboring base pairs effect the structure and/or stability of a base pair mismatch may be important to understanding the mechanisms that lead to spontaneous mutations.

In recent years, a number of investigations have examined the stability and structure of mismatched base pairs in short DNA duplexes (5–12). Nuclear magnetic resonance spectroscopy, X-ray crystallography, and UV absorbance melting studies have been employed. The stability of all mismatched bases were examined in two sequence environments (6,13,14). Results indicated that the stability and structural properties of a mismatch are influenced by its neighboring base pairs (1). No systematic study has yet to be reported on the effects of different single mismatches on the stability of long DNAs. The influence of end effects on the properties of short DNA duplexes make such a study desirable.

In addition to its interest with regard to mechanisms of spontaneous mutation, the influence of a mismatch on DNA stability is also relevant for methods that rely on thermal stability differences to separate DNAs with similar sequences. Knowledge of how a mismatch alters the stability of a DNA sequence can help optimize conditions in the selective binding of an oligonucleotide to a DNA site (15). TGGE and denaturant gradient gel electrophoresis (DGGE) are becoming widely used to detect single base mutations (16–19). The large effect of mismatched base pairs on DNA stability provides a sensitive means of detecting base pair changes (20). Understanding how a mismatch and its neighbors effect DNA stability can help identify the nature of a mutation.

In this work we have employed a vertical TGGE format (21) to determine the relative stabilities of all possible base pairs and base/base mismatches at four different positions within a 373 bp DNA. DNAs differing in thermal stability in their first melting domain unwind and decrease in mobility at different depths in a polyacrylamide gel with a superimposed temeprature gradient. In combination with site-directed mutagenesis by PCR, the temperature gradient approach provides a rapid method for examining the relative stabilities of mismatches at specific sites within a long DNA. DNAs differing in stability by 0.05 to 0.1°C were separated. Temperature gradients parallel to the direction

* To whom correspondence should be addressed

of electrophoresis were used to determine the relative stabilities of mismatches at a given site in the DNA. They provide the greatest resolution in detecting changes in DNA thermal stability. Experiments in which the temperature gradient was perpendicular to the direction of electrophoresis were used to obtain mobility transition curves.

MATERIALS AND METHODS

Materials

Taq DNA polymerase were obtained from Perkin Elmer and Promega. pUC8-31 and pUC8-36 plasmids were a gift from Dr C.Moran, Emory Univ. The plasmids contain a 130 bp segment of the *ctc* promoter region from *Bacillus subtilis* inserted between the HindIII and EcoRI sites of pUC8 (22). pUC8-31 has the wild type *ctc* sequence, and pUC8-36 has a GC to AT substitution (figure 2). DNA oligonucleotides were from Operon Inc., Alameda, CA. They were used as primers for polymerase chain reaction (PCR) amplification of the 373 bp region containing the *ctc* promoter. Sequences for the twelve upstream primers and one downstream primer are shown in figure 2. All upstream primers except UP14 were used to create a point mutation. Base positions of the mutations are underlined in the primer sequences (figure 2).

The downstream primer, designated as DP15, was end-labeled for some PCR amplifications with ^{32}P. 3 μl of γ-labeled ATP (3000 Ci/mmol, Amersham) was mixed with 1 μl (10 units) polynucleotide kinase (Promega), 1 μl 10× kinase buffer (400 mM Tris−HCl, pH 7.5, 100 mM $MgCl_2$, 50 mM DTT) and 1 μl of 10 μM primer and 4 μl of water. The mixture was incubated at 37°C for 30 mins, heated at 65°C for 5 mins and purified with a Nensorb™-20 cartridge (Du Pont).

PCR amplifications

The PCR conditions were similar to the protocol recommended by Perkin Elmer Cetus Inc. 100 μl reaction mixtures contained 50 pg of plasmid DNA, 0.6 μM of each primer, and 200 μM of each dNTP in a buffer of 10 mM Tris−HCl pH 8.3, 50 mM KCl and 2.5 mM $MgCl_2$. Reaction mixtures were overlaid with 100 μl mineral oil and thirty cycles of amplification carried out. The temperature cycles were 94°C for 1 min (except for a 4 minute first cycle), 44°C for 2 min and 72°C for 1 minute. 2−4 μl of each reaction was checked for size and purity on a 1% agarose or 7.5 % polyacrylamide gel. The PCR amplification was carried out with a 5′-end-labeled downstream primer when a labeled DNA was required. All 373 bp DNAs with or without mismatches ran with the same mobility in non-denaturing polyacrylamide gels.

TGGE

The apparatus for running the vertical temperature-gradient gel was described previously (21). A 6.5% polyacrylamide gel (37.5:1 , acrylamide:bisacrylamide) was used. The gel contained 4.2 M urea and 24% vol/vol formamide in 0.5× TBE (0.045 M sodium borate + 0.045 M Tris + 1 mM EDTA, pH 8.2). Formamide was deionized with mixed resin AG501-X8D (Bio-Rad). The gel running buffer was 0.5× TBE. Two aluminum heating blocks sandwiched the glass plates and established the temperature gradient either parallel or perpendicular to the electric field. Temperatures were measured at various positions in several test gels with a thermocouple probe (see below). The temperature gradient was linear and uniform within the region covered by the heating blocks.

The relative stability of DNAs were determined with the temperature gradient parallel to the direction of electrophoresis. DNA samples migrated from low temperature (top) to higher temperature (bottom) from 1 cm wells. For perpendicular temperature gradient gels, the DNA samples were loaded into a long well along the top of the gel. The electrophoretic direction was perpendicular to the temperature gradient. Mobility transition curves of duplex DNAs to their partially denatured states were detected as a decrease in mobility with increasing temperature (see figure 7).

Temperatures were evaluated in the gels with a needle-like thermocouple probe (TMTSS-020-6, Omega Inc.) connected to a digital thermometer (MDSD-465, Omega Inc, accuracy estimated as ± 0.1°C). Measurements were made at two position at the end of each transition run. The gels were stained with ethidium bromide and photographed. The positions where the temperature probe had been inserted were observed in the photograph and provided a temperature scale.

Photographs of the mobility transition curves were digitized and scaled using a digitizer tablet (SummaSketch II), and transmitted to a microcomputer. Transition curves were smoothed by the 'smoothlowess' function in the Axum graphics analysis package (Trimetrix Inc., Seattle, Wash.). This is based on a locally weighted regression analysis (23). The mobility transition temperature, T_u, was defined as the temperature at the peak of the derivative curve. The standard deviation of T_u measurements based on repeated experiments was 0.6°C. Differences in T_u values between two DNA transitions in a gel were reproducable within ± 0.2°C. This error estimate is based on three or four repeated measurements.

Electrophoresis conditions for several runs are described in the figure legends. In general, run times were 14 to 18 hours (overnight) at 4.5 to 6 volts/cm for the 20 cm long gels. Perpendicular temperature gradient gel results show that the 373 bp DNA melts out in several transition steps (not shown). The mobility transition temperature of the first melting domain, with Watson−Crick base pairs, is between 30 and 33.5°C under the gel conditions employed. Temperature gradients from 28.5 to 31.5°C or 28.5 to 32°C were used to optimize separation of DNAs with single base pair substitutions. A gradient from 26 to 29°C was used to optimize separation of DNAs with mismatched bases.

RESULTS

Figure 1 describes the method employed to determine the relative stability of DNAs differing by single base pairs or mismatches. Two DNAs differing by a base pair substitution were produced by PCR. One DNA was ^{32}P-labeled on the 5′-end of its downstream primer strand. The DNAs were heated for three minutes at 97°C, reannealed at 54°C for at least 10 minutes, and allowed to slowly cool to room temperature. The four resulting DNAs were analysed by parallel TGGE. Following electrophoresis, the gel was stained with ethidium bromide. DNA bands were located on a UV-transilluminator, excised, and their radioactivity measured by scintillation counting. Identities of the DNA bands were established from the radioactively labeled bands. Confirmation of band identities was made by switching the DNA that contained the labeled strand and/or by running one of the homoduplex DNAs in an adjacent lane.

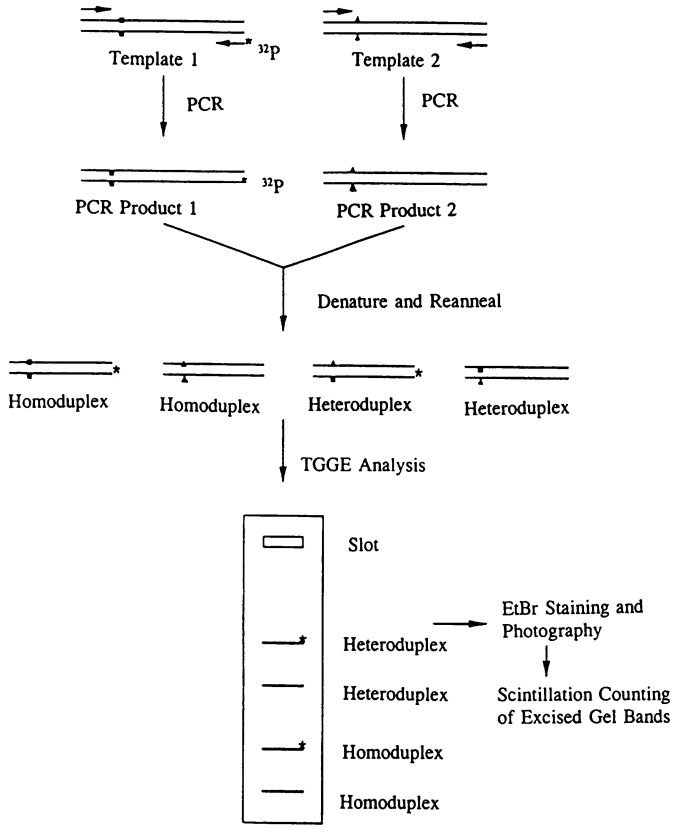

Figure 1. Protocol for determination of mismatch by PCR and TGGE. Heteroduplexes containing a single base-base mismatch were formed by melting and reannealing equal amounts of two PCR fragments, one with its downstream strand 5'-end labeled with ^{32}P. TGGE was used to separate the homoduplex and heteroduplex DNAs for analysis.

```
Upstream Primers:
      5'                                  3'
UP36C:    AATTCCATTTTTCGAGCTTTA
UP36T:    AATTCCATTTTTCGAGTTTTA
UP38T:    AATTCCATTTTTCGTTGGTTTA
UP38G:    AATTCCATTTTTCGGGGTTTA
UP38C:    AATTCCATTTTTCGCGGTTTA
UP39T:    AATTCCATTTTTCTAGGT
UP39C:    AATTCCATTTTTCCAGGT
UP39A:    AATTCCATTTTTCAAGGT
UP43G:    AATTCCATTGTTCGAGGT
UP43C:    AATTCCATTCTTCGAGGT
UP43A:    AATTCCATTATTCGAGGT
UP14:     AATTCCATTTTTCG
                  ↕   ↕↕ ↕                                    ↓
     5'   AATTCCATTTTTCGAGGTTTAAATCCTTATCGTTATGGGTATTGTTTGTAATAGGACAA
                        A (pUC8-36)
                  -43    -36                                  +1
          CTAAAACGACAAGAGGATGGTGCTGAATATGGCAACTTTAACGGCAAAAGAAAGAACGG
          ACTTTACTCGGTCGACCTGCAGCCAAGCTTGGCACTGGCCGTCGTTTTACAACGTCGTG
          ACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCC
          AGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCT
          GAATGGCGAATGGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCAC
          ACCGCATATGGTGCACTCT  3'
           3' GTATACCACGTGAGA  5' DP15 (Downstream Primer)
```

Figure 2. The 373 bp DNA sequence between the EcoRI and RsaI sites from pUC8-31 plasmid is shown. Positions −43 and −36 are indicated. The DNA fragment from the plasmid pUC8-36 has the same sequence except for a G to A substitution at position −36. The upstream primers, and the downstream primer, DP15, employed in PCR are indicated. Upstream primers created base pair changes at the positions underlined.

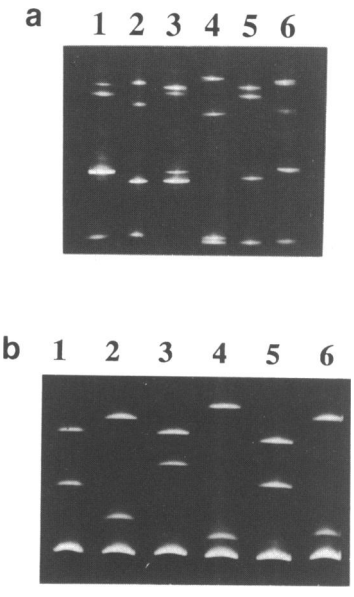

Figure 3. Parallel temperature gradient gel of 373 bp DNAs with all base pairs and mispairs at position −36. (**a**). Temperature gradient was from 28.5 to 33°C. Samples were run for 17 hrs at 90 volts. From the top to bottom in each lane DNA bands contain the following bases at position −36: 1) A·C, G·T, A·T, G·C, 2) T·C, G·A, T·A, G·C, 3) T·T, A·A, A·T, T·A, 4) C·C, G·G, G·C, C·G. 5) C·A, T·G, T·A, C·G and 6) C·T, A·G, A·T, C·G. (**b**). Temperature gradient was from 26 to 29°C. Run time and voltage were the same as above. The top two DNA bands in each each lane are the same as in (a). The lowest band in each lane contains both homoduplex DNAs, e.g., lane 1) A·C, G·T, A·T AND G·C.

The 373 bp DNAs used in the study are indicated in figure 2. PCR were used to generate thirteen DNAs differing from each other by a single base pair. The base pair changes occurred at four sites designated −36, −38, −39, and −43. This numbering scheme refers to base pair positions relative to the startpoint of transcription for the *ctc* promoter in the 373 bp DNA. Each site is located in the first melting domain of the DNA (21). Pairs of DNAs were melted and reannealed to produce 48 DNAs that contained all possible base mismatches at the four different sites each with a different base pair stacking environment.

Figures 3a and 3b show parallel TGGE experiments of 373 bp DNAs with all possible paired and mismatched bases at position −36. The nearest neighbor pairs surrounding this position are d(GXT)·d(AYC). The identity of the DNA bands in figures 3a and 3b are given in the figure caption and were based on the procedures described earlier. Figure 3a used a temperature gradient from 28.5 to 33°C to optimize the separation of base paired and mismatched DNAs in one gel. Figure 3b used a gradient from 26 to 29°C to optimize separation of the DNAs with mismatched bases. The lower temperatures of figure 3b sacrificed the ability to separate the base paired DNAs in order to determine the order of stability for all mismatched DNAs. In figure 3a, for example, it is difficult to order the relative stability of DNA bands containing A·A and T·T mismatches in lane 3 with the C·A and T·G bands in lane 5. Lane 3 of figure 3b shows a much greater separation between the DNAs with A·A and T·T mismatches. It is possible to rank their stability relative to the DNAs with C·A and T·G mismatches in lane 5.

Figures 4, 5 and 6 show parallel TGGE experiments of the 373 bp DNA with all possible paired and mismatched bases at

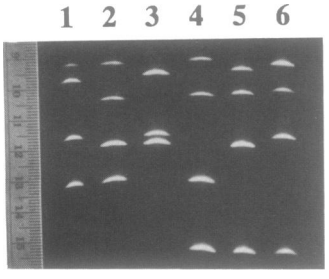

Figure 4. Parallel temperature gradient gel of 373 bp DNAs with all base pairs and mispairs at position −38. Temperature gradient was 28.5 to 33°C. From top to bottom in each lane DNA bands contain the following bases at −38: 1) A·C, G·T, A·T, G·C, 2) T·C, G·A, T·A, G·C, 3) A·A AND T·T, A·T, T·A, 4) C·C, G·G, G·C, C·G, 5) C·A, T·G, T·A, C·G, 6) C·T, A·G, A·T, C·G.

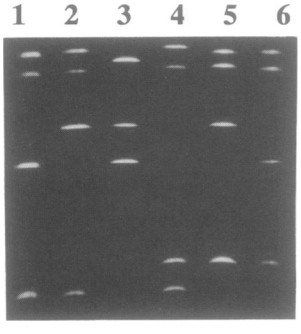

Figure 5. Parallel temperature gradient gel of 373 bp DNAs with all base pairs and mispairs at position −39, temperature gradient 29.0 to 32.5°C. From top to bottom in each lane DNA bands contain the following bases at position −39: 1) A·C, G·T, A·T, G·C, 2) T·C, G·A, T·A, G·C, 3) T·T AND A·A, T·A, A·T, 4) C·C, G·G, C·G, G·C, 5) C·A, T·G, T·A, C·G, 6) C·T, A·G, A·T, C·G.

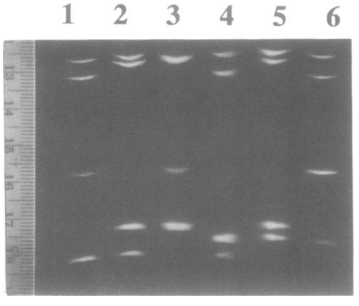

Figure 6. Parallel temperature gradient gel of 373 bp DNAs with all base pairs and mispairs at position −43, temperature gradient 29.0 to 32.5°C. From top to bottom in each lane DNA bands contain the following bases at position −43: 1) A·C, G·T, A·T, G·C, 2) T·C, G·A, T·A, G·C, 3) T·T AND A·A, A·T, T·A, 4) C·C, G·G, C·G, G·C, 5) C·A, T·G, T·A, C·G, 6) C·T, A·G, A·T, C·G.

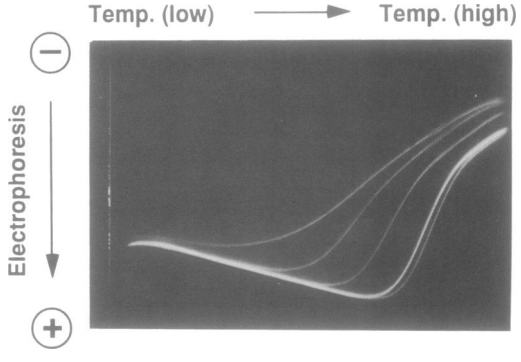

Figure 7. A typical perpendicular temperature gradient gel of 373 bp PCR fragments. Electrophoresis was conducted for 14 hrs at 90 volts. The temperature gradient was 17 to 35.5°C from left to right. A total of 3−5 μg of DNA was added across the top of the gel. The sample contained melted and reannealed DNAs with C·G and G·C at position −43, and the native DNA with T·A at the same position. The transitions from left to right correspond to DNAs with the following base pairs at position −39: C·C, G·G, T·A, C·G, G·C.

positions −38, −39 and −43 respectively. The base pairs surrounding these positions are d(GXG)·d(CYC) for −38, d(CXA)·d(TYG) for −39, and d(TXT)·d(AYA) for −43. Four bands are observed in each lane of these figures except for the third lane. The top band in the third lane of each figure contains the heteroduplex DNAs with A·A and T·T bases. A temperature gradient from 26 to 29°C was able to separate this band and show that the A·A mismatch is slightly more stable than the T·T mismatch at position −43 (not shown). This temperature gradient was unable to separate the band containing these mismatches at positions −38 and −39.

Table 1 summarizes the results from the parallel TGGE experiments. All homoduplex DNAs were more stable than their corresponding heteroduplex DNAs. This table and figures 3−6 show that both the bases at a mismatched site and the neighboring stacking interactions influence the destabilization caused by a mismatch. The least destabilizing base in a mismatch is G and the most destabilizing base to have in a mismatch is C. A similar observation was made in a systematic study of mismatches in the oligomer $d(CT_3XT_3G)\cdot d(CA_3YA_3G)$ (6). In general our results show that purine·purine (both homo- and hetero-) mismatches are more stable than pyrimidine·pyrimidine mismatches.

The most stable mismatch is different for different nearest-neighbor environments. G·T is the most stable mismatch for positions −39 and −43. G·A and G·G are the most stable mismatches for positions −38 and −36 respectively. NMR and X-ray crystallographic studies indicate that both G·T and G·A pairs can form two hydrogen bonds and stack within a DNA B-conformation duplex with relatively little distortion (1). Studies on DNA duplex oligomers with G·A mismatches indicate that intrahelical base pairing occurs but that the nature and extent of helix distortion is strongly sequence dependent (7,24). Our results confirm a sequence dependence on the properties of the G·A mismatch.

Results from the TGGE studies are in good agreement with available data on mismatch stabilities in short DNA oligomers. NMR studies (24) on d(CGXGAATTCYCG) where X·Y formed the pairs T·G, A·G, C·A, or C·T indicated an order of stability of T·G > A·G > C·A > C·T. We observed the same heirarchy in the equivalent nearest neighbor environment at position −38 (Table 1). UV absorbance melting curves were obtained by Aboul-ela *et al.* (6) from the oligomers $d(CT_3XT_3G)\cdot d(CA_3YA_3G)$ and by Gaffney and Jones (14) from the

Table 1. Comparison of Watson–Crick and mismatched base pair stabilities

Base Pair Position	5' Flanking Base Pair	3' Flanking Base Pair	The Ranking of Stability of Normal and Mismatched Base Pair
-36	G·C	T·A	C·G > G·C > T·A > A·T > G·G > A·G > G·A > G·T > T·G > A·A > C·A > T·T ≥ A·C > T·C ≥ C·T > C·C
-38	G·C	G·C	C·G > G·C > T·A > A·T > G·A > G·G > T·G > A·G > G·T > A·A = T·T > C·A > C·T, A·C > T·C > C·C
-39	C·G	A·T	G·C > C·G > A·T > T·A > G·T > G·A > G·G > A·G ≥ T·G > A·A = T·T > A·C > C·A, T·C, C·T > C·C
-43	T·A	T·A	G·C > C·G > T·A > A·T > G·T > G·G, A·G > G·A, T·G > A·A > T·T > A·C > T·C > C·C, C·A, C·T

oligomers $d(G_2T_2XT_2G_2)\cdot d(C_2A_2YA_2C_2)$ with X and Y substituted by all four DNA bases. Both studies had a similar heirarchy of stability for mismatched DNAs. The Gaffney and Jones work yielded the following order of stabilities based on T_{max}, the peak of the derivative melting curves: G·T > G·G = A·G > T·G > G·A = T·T > T·C > A·C > C·T > A·A > C·A > C·C. Table 1 shows a similar but not identical rank order of stability at position −43. The most significant difference is the relative ranking of A·A which is more stable in the DNA fragment than in the oligomers. The differences are not due to mobility differences of mismatched DNAs. In the absence of temperatures sufficient for melting, all DNA fragments have the same mobility. This is best illustrated in the perpendicular gels such as figure 7. Prior to the onset of melting the DNAs had the same mobility. Potential causes for the differences are discussed below.

Table 1 also provides direct information on the relative stabilities of Watson–Crick base pairs in four stacking environments inside a long DNA. DNAs with G·C or C·G base pairs were more stable than those with A·T or T·A pairs in all cases examined. The relative stability of several base pair stacking interactions was consistent with observations from DNA polymer melting studies. Solution studies have shown that poly (dA)·poly (dT) is more stable than poly (dAT)·poly (dAT), and poly (dGC)·poly (dGC) is more stable than poly (dG)·poly (dC) (25). These observations are consistent with the greater stability of the d(TTT)·d(AAA) sequence at position −43 relative to d(TAT)·d(ATA), and the enhanced stability of d(GCG)·d(CGC) at position −38 relative to d(GGG)·d(CCC). Similarly, the greater stability of the d(GTG)·d(CAC) sequence at position −38 when compared to d(GAG)·d(CTC) is consistent with the higher T_m of poly (dGT)·poly (dAC) when compared to poly (dGA)·poly (dTC) (25). The above agreement can not be automatically expected since the solvent employed in TGGE differs from salt solutions commonly used in UV absorbance melting studies.

Perpendicular TGGE experiments display the mobility transitions of intact double-stranded DNA to the denatured state. Figure 7 shows transition curves of five of the DNAs examined. The initial increase in mobility with increasing temperature prior to the main transition is due to the effect of temperature on the gel. The sigmoidal decrease in mobility is due to the unwinding of DNA strands. The temperature range was selected to include the first melting domain of the 373 bp DNAs. The two leftmost transitions in figure 7 correspond to DNAs contain C·C or G·G mismatches at position −39. The middle transition corresponds to the DNA with the T·A base pair at position −39. The two closely spaced transitions on the right correspond to DNAs with C·G and G·C base pairs at position −39. The curve of the C·C mismatch DNA is much broader then the other transitions. This observation was common for the transitions involving DNAs with mismatched base pairs of low stability. An analogous observation was made by UV absorbance melting studies of DNA oligomers with mismatches (13), and may indicate that internal melting plays a significant role in the melting process.

Table 2. Perpendicular TGGE [a]T_u measurements of 373 bps DNA first melting domain

-36 set (°C)		-38 set (°C)		-39 set (°C)		-43 set (°C)	
C·G:	31.8	C·G:	33.4	[b]G·C:	31.8	G·C:	32.4
[b]G·C:	31.8	G·C:	32.6	C·G:	31.6	C·G:	31.9
T·A:	31.0	T·A:	32.1	A·T:	30.6	[b]T·A:	31.8
A·T:	30.9	[b]A·T:	31.8	T·A:	30.1	A·T:	31.2
G·G:	29.6	G·A:	30.6	G·T:	28.8	G·T:	29.8
A·G:	29.5	G·G:	30.4	G·A:	28.8	G·G:	29.7
G·A:	29.4	T·G:	30.3	G·G:	28.7	A·G:	29.7
G·T:	28.8	A·G:	30.1	A·G:	28.6	G·A:	29.5
T·G:	28.5	G·T:	29.8	T·G:	28.6	T·G:	29.5
A·A:	28.3	A·A:	29.5	A·A:	28.5	A·A:	29.3
C·A:	28.1	T·T:	29.5	T·T:	28.5	T·T:	29.3
T·T:	28.0	C·A:	29.4	A·C:	28.4	A·C:	29.2
A·C:	28.0	C·T:	29.0	C·A:	28.2	T·C:	29.2
T·C:	27.9	A·C:	29.0	T·C:	28.2	C·C:	29.1
C·T:	27.9	T·C:	28.7	C·T:	28.2	C·A:	29.1
C·C:	27.9	C·C:	28.5	C·C:	28.1	C·T:	29.1

[a] Tu was 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. Each experiment contained the pUC8-31 DNA fragment as an internal standard. The mean Tu of this DNA was 31.8°C (± 0.6°C) based on 19 repeated experiments.
[b] This is the same pUC8-31 DNA fragment.

Table 3. Comparison of DNA destabilization by single-base mismatch in different nearest-neighbor environments

Base Pair Position	DNA SEQUENCE	*ΔT_u (°C)	
		T_u(G·C)-T_u(C·C)	T_u(G·C)-T_u(G·G)
-36	d(GXT)·d(AYC)	3.9	2.2
-38	d(GXG)·d(CYC)	4.1	2.2
-39	d(CXA)·d(TYG)	3.7	3.1
-43	d(TXT)·d(AYA)	3.3	2.7

* T_u differences are obtained by subtracting T_u values of DNA duplexes with C·C or G·G mismatch from T_u values of DNA duplexes with G·C base pair at the same position.

Transition temperatures, T_u, for transitions of all of the base paired DNAs and DNAs with mismatches are given in Table 2. T_u values of DNA molecules with a single mismatch are lower than the corresponding values for homoduplex DNAs by 1 to 5°C. The order of stability for each position is based on results from Table 1 since the resolution of the parallel gradient gel was better than perpendicular temperature gradient gels. All of the transition curve data were consistent with the results from the parallel TGGE. Table 3 compares the destabilization caused by two different base changes in the four nearest neighbor environments. It illustrates that the destabilization caused by a mismatch depends on its nearest neighbor base pairs. Converting a G·C base pair to a C·C has its largest effect in the d(GXG)·d(CYC) environment. Changing a G·C to a G·G has its largest effect in the d(CXA)·d(TYG) environment.

DISCUSSION

TGGE provides a rapid method to characterize the relative stability of different base pairs and mismatched bases at specific sites within a DNA molecule. Agreement is observed between the heirarchy of Watson–Crick base pair stacking interactions from TGGE and DNA polymer melting studies. The presence of urea and formamide does not appear to cause a major alteration in the heirarchy of base pair stacking interactions. This is consistent with previous melting studies that indicate urea and formamide lower the thermal stability of GC and AT base pairs by approximately equivalent amounts (16,26,27,28,29).

The heirarchy of mismatch stabilities in Table 1 is also in relatively good agreement with data available from DNA oligomer melting studies in aqueous solutions. For the d(GXG) environment the heirarchy of stability is the same as that observed for four mismatches studied in a DNA oligomer (24). The most stable and least stable mismatches we observe for the d(TXT)·d(AYA) environment are also among the most and least stable mismatches observed in DNA oligomer melting studies (6,14). However, as described earlier, the stability ranking of several mismatches in this environment differ between the fragment and oligomer data. The discrepancies do not appear to be from measurement uncertainties. TGGE indicate that the G·T mismatch is more stable than the G·G mismatch. This agrees with the 2.3°C separation in melting curves of related DNA oligomers (14). Yet TGGE can not separate DNA fragments with G·A and T·G mismatches, although DNA oligomer melting studies show the T·G mismatch to be more stable than G·A by 2.8°C (6,14).

Several possible causes exist for the descrepancies in the mismatch rank order between the oligomer and fragment data for d(TXT)·d(AYA). An obvious possibility is the difference in solutions employed. The urea-formamide solution may alter the relative stability of some mismatches when compared to NaCl solutions used for the oligomer studies. Another consideration is the oligomer vs. polymer contexts of the studies. Different mismatches may differentially influence the duplex-strands dissociation step which is assumed to be a two state process for all oligomers. This step dominates DNA oligomer melting. It is absent in the unwinding of a fragment's first melting domain.

The influence of the twelve possible mismatched bases on DNA stability varies with nearest neighbor environment. For the four sites examined G·T, G·G, and G·A pairs are always among the most stable mismatches, and the pyrimidine·pyrimidine mismatches are among the least stable. However the specific mismatch that creates the most or least instability depends on the neighboring sequence (Table 3). Results from this and related work should be of value for methods that utilize DNA duplex formation for sequence-specific recognition. Competitive oligonucleotide priming (15) and related methods require conditions that maximize the difference in stability between a completely complimentary DNA duplex and a duplex with one mismatch. Results from this study may also be of value in characterizing a base pair substitution detected by TGGE or DGGE. The pattern of bands produced by melting and reannealing two DNA molecules differing by a single base pair substitution depends on the base pair change and its nearest neighbor pairs. One may be able to characterize the base pairs neighboring the mutant site as well as the type of base pair change that has occurred.

This study illustrates the ressolving power of vertical TGGE and its sensitivity to the temperature gradient employed. The most effective gradient in our parallel TGGE experiments was 3 to 4°C. This gradient was spread over the 15 cm height of the heating blocks. One can readily distinguish DNA bands separated by 2 mm. Thus the parallel TGGE experiment can separate DNAs differing in stability by 0.05°C. Figures 3a and 3b demonstrate how changes in the midpoint temperature of the gradient allow for the separation of DNA bands differing by a base pair or by a mismatch. In our experiments samples of melted and reannealed DNAs differing by a single base pair substitution produced three to four bands for gradients of 8°C or less. The temperature range selected was an important parameter in determining which bands separated.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the technical assistance of Thomas Maier in the physics department of Georgia Tech. This work was supported by N.I.H. grant GM38045.

REFERENCES

1. Modrich,P. (1987) *Annu. Rev. Biochem.*, 56, 435–466.
2. Akiyama,M., Maki,H., Sekiguchi,M. and Horiuchi,T. (1989) *Proc. Natl. Acad. Sci. USA.*, 86, 3949–3952.
3. Singer,B., Chavez,F., Goodman.M.F., Essigmann,J.M., Dosanjh,M.K. (1989) *Proc.Natl.Acad. Sci. USA*, 86, 8271–8274.
4. Blake,R.D., Hess,S.T. and Nicholson-Tuell,J. (1992) *J. Mol. Evol.* 34, 189–200.
5. Brown,T., Leonard,G.A., Booth,E.D. and Kennard,O. (1990) *J. Mol. Biol.*, 212, 437–440.
6. Aboul-ela,F., Koh,D., Tinoco,I.Jr., and Martin,F.H. (1985) *Nucleic Acids Res.*, 13, 4811–4824.
7. Brown,T., Hunter,W.N., Kneale,G.G. and Kennard,O. (1986) *Proc. Nat. Acad. Sci. USA*, 83, 2402–2406.
8. Gao,X. and Patel,D.J. (1988) *J. Am. Chem. Soc.*, 110, 5178–5182.
9. Patel,D.J., Kozlowski,S.A., Marky,L.A., Rice,J.A., Broka,C., Dallas,J., Itakura,K. and Breslauer,K.J. (1982) *Biochemistry* 21, 437–444.
10. Roongta,V.A., Jones,C.R. and Gorenstein,D.G. (1990) *Biochemistry*, 29, 5245–5258.
11. Kneale,G., Brown,T., Kennard,O. and Rabinovich,D. (1985) *J. Mol. Biol.*, 186, 805–814.
12. Arnold,F.H., Wolk,S., Cruz,P. and Tinoco,I.J. (1987) *Biochemistry*, 26, 4068–4075.
13. Werntges,H., Steger,G., Riesner,D. and Fritz,H-J. (1986) *Nucleic Acids Res.*, 14, 3773–3790.
14. Gaffney,B.L. and Jones,R.A. (1989) *Biochemistry*, 26, 5881–5889.
15. Gibbs,R.A., Nquyen,P-N., Caskey,C.T. (1989) *Nucleic Acids Res.*, 17, 2437–2448.
16. Lerman,L.S., Fischer,S.G., Hurley,I., Silverstein,K. and Lumelsky,N. (1984) *Annu. Rev. Biophys. Bioeng.*, 13, 399–423.

17. Guldberg,P. and Guttler,F. (1993) *Nucleic Acids Res.*, 21, 2261–2262.
18. Ke,S-H., Kelly,P.J., Wartell,R.M., Hunter,S.H. and Varma,V.A. (1993) *Electrophoresis*, **14** 561–565.
19. 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.
20. Abrams,E.S., Murdaugh,S.E., and Lerman,L.S., (1990) *Genomics* 7 463–475.
21. Wartell,R.M., Hosseini,S.H., and Moran,J.D. (1990) *Nucleic Acids Res.*, 18, 2699–2705.
22. Tatti,K.M. and Moran,C.P.Jr. (1985) *Nature*, 314, 190–192.
23. Cleveland,W.S. and Devlin,S.J. (1988) *J. Amer. Stat. Assoc.*, 83, 596–610.
24. Patel,D.J., Kozlowski,S.A., Ikuta,S. and Itakura,K. (1984) *Federation Proc.*, 43, 2663–2670.
25. Wells,R.D., Wartell, R.M. (1974) In Burton, K.(ed.), Biochemistry of Nucleic Acids. Butterworth & Co., London, Vol. 6, pp. 41–64.
26. Nishigaki,K., Husimi,Y., Masuda,M., Kaneko,K. and Tanaka,T. (1984) *J. Biochem.*,95, 627–635.
27. Hutton, J. R. (1977) *Nucleic Acids Res.*, 4, 3537–3555.
28. Klump,H., and Burkart,W. (1977) *Biochim. Biophys. Acta*, 475, 601–604.
29. Casey,J. and Davidson,N. (1977) *Nucleic Acids Res.*, 4, 1539–1552.