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THE EFFECTS OF UNPAIRED BASES IN AN OLIGO DEOXY (A) * DEOXY (T) TRACT ON DNA BENDING

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THE EFFECTS OF UNPAIRED BASES IN AN OLIGO DEOXY(A)•DEOXY(T) TRACT ON DNA BENDING

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Abstract

DNA bending in oligonucleotides has been studied based on anomalous gel migration of DNA sequences containing the oligo d(A)•d(T) tract and unpaired bases. The oligo d(A)•d(T) tract or the A-tract contains adenine repeated four or more times in succession. Studies have shown that an A-tract in a DNA sequence causes a bend in the DNA helix. Two models have been set forth to explain the structural basis of DNA bending, the wedge model and the junction bending model. It has been shown that both the oligo d(A)•d(T) tract and unpaired bases cause a bend in the DNA helix. Stacking interactions of unpaired bases with their neighboring base pairs as well as the size of the nucleotide are important in determining the bending caused by unpaired bases. This study focuses on the effects of unpaired bases in the middle of the oligo d(A)•d(T) tract.

Introduction

Background

DNA curvature was first observed in conserved sequences of trypanosome kinetoplast DNA (kDNA) (Hagerman, 1986). Kinetoplast DNA is the mitochondrial DNA found in parasitic protozoans (Marini et al., 1984). The kDNA sequences displayed significant anomalous gel migration. The curvature was caused by adenine repeated four or more times in a series (Searle et al., 1990). It was this initial discovery that has led to much research into the structure and function of the oligo d(A)•d(T) tract.

Curved DNA is also present in other species including humans. It is thought to be functionally important in replication, transcription and recombination (Hagerman, 1990). It is most often present in untranslated segments of the genes, such as promoter regions (VanWye et al., 1991). Curved DNA is present at the origin of replication in bateriophage lambda and serves a function in protein recognition (Hagerman, 1990). In *E. coli*, DNA bending is proportional to the number of T_5 and T_6 tracts present at the replication origin. Substitution of these bases leads to a loss in promoter activity (Lozinski et al., 1991). DNA-protein interactions include complexes with repressors, histone octamers, transcription and recombination. Multiple copies of a serine-proline-lysine-lysine (SPKK) peptide

sequence present in tails of certain histone H_1 bind to the narrow minor groove of DNA sequences with an oligo d(A)•d(T) tract. This serves as a global recognition structure and stabilizes the bend in the DNA (Travers, 1990). Hydrogen bond donors and acceptors on the bases allow recognition of DNA by proteins without unwinding the DNA (Trifonov, 1991). Binding of proteins involved in gene regulation may trigger bending in the helix which is not in the linear "B" form (Chan et al., 1990). Some sequences are bent to accommodate protein recognition and binding while other sequences bend further upon binding (Chan et al., 1990).

Furthermore, nuclease digestion experiments have shown that promoter regions of genes and origins of replication are very sensitive to nucleases. Nucleosome cores form a complex in which the DNA is tightly bent (Travers, 1990). The lack of nucleosomes at this site allows RNA polymerase to gain easy access to these sites and begin transcription (Losa et al., 1990).

Another area that has gained much attention in recent years is the interactions of drugs with bent DNA. Distamycin, an antitumor drug, binds in the minor groove of A•T rich regions through hydrogen bonding, Van der Waals and electrostatic interactions (Martello et al., 1989 and Coll et al., 1987). The pyrrole rings interact with the planar amides which allow the drug to take on a "crescent shape". This shape, similar to the curve in the minor groove of B-DNA, allows the binding of the drug to the DNA. The amino

group on the N-2 of guanine prevents the drug from binding to G•C rich regions (Coll et al., 1987). It has been proposed that the drug has an affinity for the A•T base pairs in the narrow minor groove because it is devoid of N-2 amino groups found with guanines. When distamycin binds to the A-tract, it eliminates the curvature which is important for cell division to occur (Coll et al., 1987).

Direction of curvature is also important so that models for protein induced bending can be devised. For example, a nicking enzyme in *C. fasciculata* binds DNA which is curved. This binding is critical to replication of kinetoplast minicircles. The nicking enzyme was allowed to bind to a plasmid to determine if the structure of DNA is necessary for recognition. It was observed that plasmids that had curved sequences were substrates for the nicking enzymes. When the plasmid is in the supercoiled state, the enzyme binds to curved and non-curved DNA; however, in the linear form, it only binds to a plasmid with curved DNA. Kinetoplast DNA in the presence of distamycin has reduced curvature. Therefore, enzyme activity is reduced. As the concentration of distamycin is increased, the enzyme does not bind DNA (Kitchin et al., 1986). Thus, the sequence itself is not needed for enzyme recognition, but the altered DNA helix is necessary (Kitchin et al., 1986).

The Oligo d(A)•d(T) Tract

Numerous studies have shown that the presence of an A-tract in a DNA sequence causes a bend in the DNA helix. Two models have been proposed to explain the bending in the A-tract. The first proposal suggests that neighboring base pairs are not parallel, thus creating a wedge between the nucleotides resulting in a kink in the DNA helix. In order for the bend to become additive, the deflection caused by each wedge has to be in the same direction and the wedge has to be in phase with the helix axis (Trifonov, 1991). The wedge is created within the A-tract by N-6 amino group of adenines which are juxtaposed (Trifonov, 1991). The wedge model assumes that the wedge between all dinucleotides is the same (Hagerman, 1990).

One criticism against the wedge model was demonstrated through gel electrophoresis experiments. It was discovered that sequences of the series, 5'-$[GA_4T_4C]_N$-3' migrated much more slowly than its counterpart, 5'-$[GT_4A_4C]_N$-3' implying that polarity of the A-tract was significant in DNA bending (Hagerman, 1986). Imino-proton-exchange NMR experiments demonstrated that exchange times of GGA_3T_3CC were five times longer than those of CCT_3A_3GG. This led to the conclusion that oligonucleotides with long lifetimes must have an A-tract in the A ⟶ T orientation rather than in the T⟶ A orientation in order to exhibit long exchange times (Sarma et al., 1988 and Gupta et al., 1988). The other argument

against the wedge model is that the susceptibility of an A-tract to hydroxyl radical cleavage depends on polarity. The cleavage pattern for A_4T_4 is different than the rest of the B-DNA. For T_4A_4, the cleavage pattern is similar to the rest of the B-DNA (Hagerman, 1990).

The second model for DNA bending states that two straight helices can create a distortion of the helix axis at the junction. This is done to ensure proper base stacking interactions at the junction (Hagerman, 1990). When the junctions are in phase, the curvature becomes additive (Nadeau et al., 1989). Fiber diffraction studies indicate that in the junction bending model, the conformation of the A-tract is similar to poly (dA) • poly (dT) structure (Nadeau et al., 1989). The poly (dA) • poly (dT) structure is a modified B-form DNA which cannot undergo a B-form to A-form transition (Brahms et al., 1990). When a B-DNA segment is placed next to the tilted bases of the A-tract, the helix is deflected at the junction which creates a bend in the junction. The longer the A-tract, the more easily it can overcome the free energy barrier and assume the altered conformation which bends. At high temperatures, the equilibrium between the B-form and the alternative form of the oligo d(A)•d(T) tract is shifted towards the B-form. As a result, the A-tract bending decreases at high temperatures (Koo et al., 1986).

Furthermore, changes in the structural conformation of the A-tract lead to curvature of the DNA helix. Adenine and

thymine bases in A-tract DNA display a high degree of propeller twist (DiGabrielle et al., 1989). Propeller twists rotate the bases along the longitudinal axis (Nelson et al., 1987). A high degree of propeller twist leads to the stability of the helix by maximizing purine-purine interactions and allowing the formation of more hydrogen bonds. The amino group at C-6 of adenine is forced towards the oxygen at C-4 of thymine due to propeller twisting. This leads to the formation of a non-Watson-Crick hydrogen bond across the major groove along with Watson-Crick hydrogen bonds (Nelson et al., 1987). The stacking of adenines occurs by overlapping of its 6-membered rings. For the thymine bases, the C-5 methyl groups of thymine and the C-6 atoms of the pyrimidine ring below it undergo Van der Waals interactions. The formation of non-Watson-Crick hydrogen bonds and base stacking interactions leads to an increased helical twist and a decreased helical rise of 3.2Å, instead of 3.4Å (Nelson et al., 1987). These interactions also lead to the narrowing of the minor groove in A•T rich DNA sequences (Nelson et al., 1987).

Curvature is eliminated when an A-tract is interrupted with a guanine residue, indicating that purine residues are not equivalent. Interruption of the A_5T_5 tract with a G•C base pair leads to the loss of curvature. For example, the addition of the guanine residue in the A-tract does not exhibit unusual exchange rates in imino-proton-exchange NMR experiments. The amino group of guanine at C-2 does not

allow bending to occur by preventing the reduction of the minor groove. However, when an I•C base pair is substituted, it retains the same degree of curvature as the A_5T_5 tract. Furthermore, AAIAA has only a slight loss of long exchange times seen in A_5T_5. The presence of an amino group at the C-6 position and the absence of a substituent at the C-2 position of adenine are requirements for the existence of DNA curvature (Hagerman, 1990).

Anomalous Migration of A-tract DNA in Polyacrylamide Gels

Polyacrylamide gel electrophoresis is used to study the anomalous migration of the oligo d(A)•d(T) tract. Migration at high temperatures is more like regular B-DNA due to the shift in equilibrium from the alternative form to the B-form DNA (Abagyan et al., 1990). Mobility of the DNA varies with the location of the bend (Hubner et al., 1989). Permutation methods which are used to identify and localize curvature indicate that if the bend is in the middle of the fragment, it is harder for the sequence to migrate through the gel matrix (Hagerman, 1990).

Adenine tracts with three or less adenine residues show very little anomalous behavior. Four or more adenines in the A-tract exhibit significant anomalous behavior. An A-tract with six adenine residues exhibits the greatest amount of curvature. Each A-tract produces a bend in the helix. When

the A-tract is repeated in phase with the helix screw, the bend becomes additive. Furthermore, as the end to end distance decreases due to an increase in the overall bend of the helix, mobility through the gel matrix decreases (Koo et al., 1988). Neighboring base pairs also affect mobility. For example, the sequence, $(GA_4T_4CC)_n$, is significantly hindered in the gel matrix; where as, $(CT_4A_4G)_n$ and $(GT_4A_4C)_n$ exhibit normal migration (Hagerman, 1990).

DNA Bending Due to Unpaired Bases

Unpaired or extra bases arise when single stranded DNA or RNA folds on itself. They are also found in double stranded DNA due to recombination or errors in replication. Extensive studies by Wang et al. (1991) have indicated that a one base bulge leads to significant anomalous migration. Two to four base bulges in one area leads to an even greater retardation in gel electrophoresis. Unpaired, intrahelical base pairs create a wedge that kinks the helix. NMR experiments have indicated that unpaired adenine residue stacks into the helix regardless of the temperature and neighboring base pairs. The same is true for unpaired thymine bases. Furthermore, an unpaired cytosine residue stacks in or loops out of the helix depending on the temperature at which the helix is present (Hseih et al., 1989, Wang et al., 1991). At 40° C, an unpaired cytosine stacks into the helix; whereas, at 0° C, it loops out of the

helix. It is expected that at 40° C, the unpaired adenine, thymine and cytosine would cause the same degree of retardation since all three unpaired bases are stacked into the helix. However, a DNA sequence with an unpaired adenine exhibited the greatest retardation of all three in a polyacrylamide gel matrix. It can be concluded that the size of the unpaired base also plays a role in determining the degree of bending in a sequence. Furthermore, it was shown that unpaired guanine and adenine exhibit the same degree of retardation while unpaired cytosine and thymine display similar anomalous migration (Wang et al., 1991). In general, purine bulges exhibit greater gel retardation than pyrimidine bulges (Wang et al., 1991), and NMR experiments have determined that purine bulges are usually intrahelical; whereas, pyrimidine bulges are usually extrahelical (Rice et al., 1989).

Neighboring base pairs also influence bending of the DNA helix caused by unpaired bases. An unpaired cytosine exhibited the greatest gel retardation when surrounded by G•C base pairs, followed by one surrounded by a mixture of bases (T•A and G•C). It displayed the least amount of retardation when flanked by T•A base pairs (Wang et al., 1991). An unpaired adenine demonstrated the greatest anomalous gel migration when flanked by G•C base pairs, followed by T•A base pairs. A mixture of base pairs around an unpaired adenine exhibited the least amount of retardation (Wang et al., 1991). LeBlanc et al. (1991) have studied the presence

of unpaired bases in an oligo d(A)•d(T) tract in sequences similar to the ones used in these experiments (Figure 1). Through UV absorption techniques, they found that unpaired bases present in the middle of the A-tract were more stable than those located in the T-tract. Unpaired bases in the A-tract that stack into the helix disrupt the pyrimidine-pyrimidine interactions on the opposite strand; however, unpaired bases in the T-tract interrupt purine-purine interactions which are harder to correct without destabilizing the helix (LeBlanc et al., 1991).

Rice et al. (1989) have performed experiments by placing one unpaired base at different distances from the A-tract for every two turns of the helix. One unpaired base every two turns of the helix exhibits low curvature. However, when an A-tract was added to the sequence, the bending caused by the bulge was enhanced (1989). This experiment demonstrated that unpaired bases in phase with the helical repeat, enhance DNA curvature (Rice et al., 1989).

Measuring the magnitude of curvature

The degree of curvature can be analyzed by using ratios of circular to linear multimers. It is based on ring closure kinetics of DNA sequences that contain A-tracts (Koo et al., 1990). This method is not very accurate because the nature of the curvature cannot be deduced. Another similar method uses ligated multimers that have been cyclized. This reveals

the degree of curvature as long as controls are used along with the A-tract DNA (Hagerman, 1990).

By far, the simplest and most popular method to study A-tract bending is to measure relative migration of the sequences in a polyacrylamide gel. Relative curvature is based on the average number of A_6-tracts per helix turn in multimers (Koo et al., 1986). Anomalous gel migration is measured as ratio of the apparent length of the DNA fragment divided by the actual length of the sequence (R_L). The ratio of the lengths is equal to one for normal gel mobility (Koo et al., 1990). A value of R_L greater than one indicates bending of the DNA helix axis (Hagerman, 1990).

In these experiments, I have determined the effects of unpaired bases in an oligo d(A)•d(T) tract on DNA bending through the use of polyacrylamide gel electrophoresis. The local structures of sequences similar to the ones used here have been determined by nuclear magnetic resonance spectroscopy by Morden et al. (1990, 1993). In addition, UV absorbance techniques have been used to determine the stability of unpaired bases in the A-tract as discussed before (LeBlanc et al., 1991). The bending studies were performed to determine the global structural properties of the DNA helix since the local structures have already been discerned. Previous studies conducted in this field have made assumptions about the local structure of their sequences based on NMR studies performed on comparable sequences. It has been shown that an oligo d(A)•d(T) tract and unpaired

bases cause a bend in the DNA helix. The presence of an unpaired base in the middle of the A-tract can increase the bend caused by the A-tract or diminish it. Unpaired purine bases cause a greater degree of retardation than unpaired pyrimidine bases (Hseih et al., 1989 and Wang et al., 1991). The ratio of the apparent length of the sequence was compared to the actual length of the DNA sequence to determine the presence or absence of increased bending due to the presence of unpaired bases in the A-tract. The sequences used in these experiments are shown in Figure 1.

```
5'          A-T-G-T-G-C-G-A-A-A-A-G-C-G-A-C-T-C-G-G-C   3'
3' G-C-C-G-T-A-C-A-C-G-C-T-T-T-T-C-G-C-T-G-A           5'
```

Perfect Duplex

```
5'          A-T-G-T-G-C-G-A-A---A-A-G-C-G-A-C-T-C-G-G-C   3'
3' G-C-C-G-T-A-C-A-C-G-C-T-T-G-T-T-C-G-C-T-G-A           5'
```

T-Strand G-bulge
The bulge is extrahelical

```
5'          A-T-G-T-G-C-G-A-A-G-A-A-G-C-G-A-C-T-C-G-G-C   3'
3' G-C-C-G-T-A-C-A-C-G-C-T-T---T-T-C-G-C-T-G-A           5'
```

A-Strand G-bulge
The bulge is intrahelical

```
5'           A-T-G-T-G-C-G-A-A---A-A-G-C-G-A-C-T-C-G-G-C   3'
3'  G-C-C-G-T-A-C-A-C-G-C-T-T-C-T-T-C-G-C-T-G-A          5'
```

T-Strand C-bulge
The bulge is extrahelical

```
5'          A-T-G-T-G-C-G-A-A-G-A-A-G-C-G-A-C-T-C-G-G-C   3'
3' G-C-C-G-T-A-C-A-C-G-C-T-T-C-T-T-C-G-C-T-G-A           5'
```

Interrupted A- and T-tracts

Figure 1. Sequences used in the DNA bending experiments. The structure of the sequences were determined by NMR experiments in the Morden lab.

Materials and Methods

Oligonucleotide Synthesis and Measurement of Absorbances

Synthesized oligonucleotides were purchased from Midland Certified Reagent Company (Lot# 081093-330, -327, 101493-242, 102093-332 and -334). The extinction coefficients of the oligonucleotides were calculated by the nearest neighbor analysis. The spectrophotometer was used to determine the absorbance at 260 nm. The A_{260} was used to calculate the amount of oligonucleotide needed for the phosphorylation reactions.

Phosphorylation of the Double Stranded DNA

The single stranded DNA was combined with its complementary strand and heated to 85-94° C to yield a 5 ODU reaction mixture. The mixture was allowed to cool to room temperature and further cooled in the refrigerator to 4-5° C before phosphorylation. The double stranded DNA was then phosphorylated at the 5' ends by combining the oligonucleotides with polynucleotide kinase buffer (70 mM Tris-HCl, 10 mM $MgCl_2$, 5 mM DTT at pH 7.6), 10 mM ATP and T4 polynucleotide kinase (New England Biolabs). The reaction mixture was then placed in a 37° C water bath for 45 minutes. Additional kinase was added to the sample which was incubated for another 45 minutes.

Ligation of the DNA Duplexes

A ligation reaction with ~0.4 ODU of the phosphorylated DNA duplex, T4 DNA Ligase (New England Biolabs) and 1X T4 DNA ligase buffer (50 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP and 50 µg/ml BSA) was performed at 4-5° C. Several ligation reactions were run for different periods of time for each duplex to determine the optimal ligation time for each molecule. The ligation reaction was quenched by adding loading solution which contained EDTA (Sigma).

Gel Electrophoresis

The samples were run on 12% polyacrylamide gels [19:1 acrylamide: bis(acrylamide) ratio; 15 cm X 15 cm X 1.5 mm]. with 1X TBE solution as running buffer. Each well was loaded with ~0.2 ODU of the ligated product. The gels were run at room temperature at 10-11 V/cm for 4-8 hours. Bromophenol blue in the loading solution was used as a tracking dye to assess the progress of the gel. DNA restriction fragments from a pBR322 Hae III digestion were used as molecular weight markers to which the gel retardation of the DNA multimers was compared. The relevant size markers in the restriction digests were 21, 51, 57, 64, 80, 89, 104 and 123 base pairs in length.

Visualization of the DNA Multimers

The gels were stained in 1X TBE and 1 µg/ml ethidium bromide solution for 20-25 minutes to allow the DNA to become

stained. They were destained in 1X TBE for 30 minutes The DNA was visualized with the use of a UV lamp. The gel was photographed on Polaroid Type 667 film.

Calculating the Ratio of the Length (R_L) of the Sequences

The distance traveled by each band from the bottom of the well was measured using a ruler. The migration of the molecular weight marker was taken as a standard against which all the DNA bands were analyzed. Since the molecular weight marker does not have bands corresponding to the multimers observed in the DNA sequences used, a graph of 1/mobility versus the number of base pairs was constructed for the molecular weight marker (Figures 4 and 6) (Rickwood, 1982). The equation of this line was used to calculate the theoretical migration of base pairs corresponding to the multimers. The ratio of the apparent length of the DNA sequence to the actual length of the sequence (R_L) was calculated to determine the presence of bending in the sequences.

Results and Discussion

The optimum ligation times have been determined for all the oligonucleotides used in these experiments (See Table I, page 21). Ligation reactions run on 12% gels are depicted in Figures 2 and 3. A ligation ladder is visible for all the sequences present in the gel. Measurements made based on the migration distances for each multimer and the restriction fragments from a pBR322 Hae III digestion were used to calculate the ratio of the lengths (R_L) (Figures 4 and 6). As seen on the graph in Figure 5 and 7, the T-strand G-bulge displayed the greatest anomalous migration followed by the T-strand C-bulge, the A-strand G-bulge, and finally the perfect duplex. It is apparent from these results that the presence of an unpaired base in the middle of the oligo d(A)•d(T) tract does enhance the curvature caused by the A-tract. The duplex with the interrupted A- and T-tracts also exhibited bending comparable to the bulged sequences.

The ratio of the lengths of the sequences used in these experiments is small compared to those in the literature. One possibility is that since these sequences contain one A-tract and as a result, one unpaired base every two turns of the helix the bending present is not optimal. If an A-tract was present for every turn of the helix, the bending might be increased. I have performed experiments with sequences that were 10 base pairs in length having an A-tract every turn of the helix; however, these sequences did not ligate well

because the unpaired bases were located too close to the ends of the sequence. Furthermore, the 21 base pair sequences used in the experiments contain an A4-tract which is the minimum amount of adenine residues required to observe bending. Another possibility is that the measurement of the bands at the top of the gels are inaccurate because the longer sequences were not separated adequately. Finally, error can be introduced when using the standard curves in determining the theoretical mobility of the ligated sequences.

I have encountered several problems with the ligation reactions and the gels. First, I have noticed that the ligation reactions have not been reproduced efficiently. I performed one larger ligation reaction to alleviate this problem. However, the presence of exonuclease activity in the ligation reactions has curtailed the use of the ligated products after a period of one week. Furthermore, the bands in the gels are diffuse. Running the gels at a higher voltage caused an increase in the temperature of the gel matrix which resulted in an uneven migration of the samples in each lane. Changing the percent composition of the acrylamide in the gels did not alleviate the problems of resolving the longer DNA sequences or the diffusion of the bands.

In the future, the gels have to be run at lower temperatures (i.e. 4° C room) in order to alleviate the problems caused by heating. Moreover, higher voltages have

to be used in order to limit the diffusion of the bands. The ligation reactions will be ethanol precipitated to obtain sharper bands and reduce the exonuclease activity. Furthermore, a 21 base pair random sequence will be used to directly determine the relative migration of the bent sequences.

Table I.

DNA Duplex	Ligation Time (Hours)
A_4/T_4	3
A_2GA_2/T_4	4.5
T_2GT_2/A_4	3.5
T_2CT_2/A_4	4
A_2GA_2/T_2CT_2	3.5

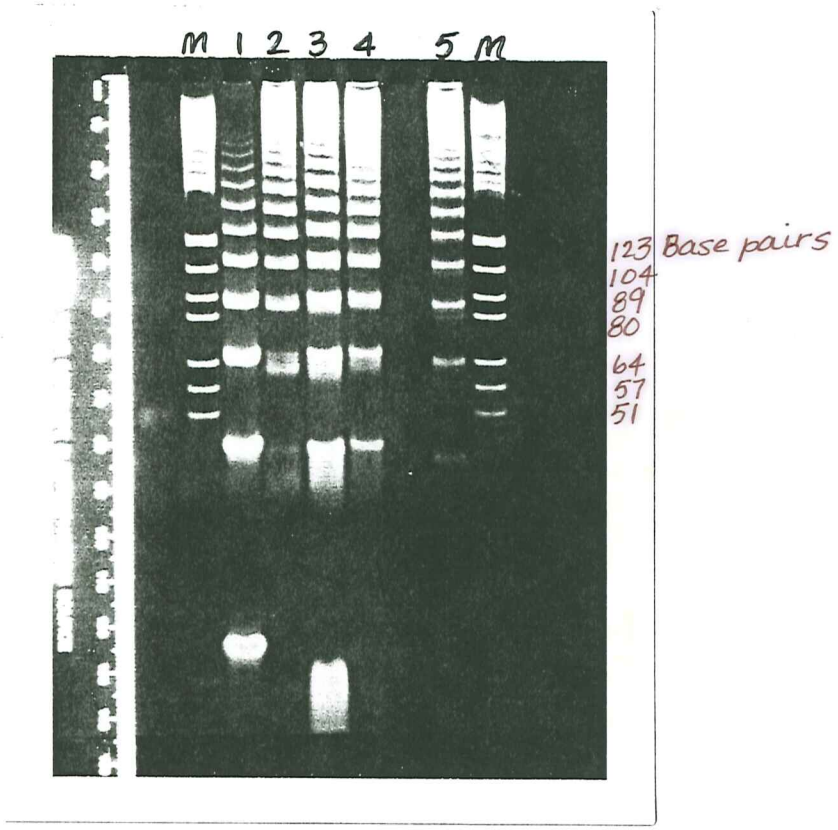

Figure 2: An ethidium bromide stained gel with all the ligated products and the molecular weight marker, pBR322 Hae III digest. Lane 1 contains the interrupted A- and T-tracts (A_2GA_2/T_2CT_2), lane 2, the T-strand C-bulge (T_2CT_2/A_4), lane 3, the T-strand G-bulge (T_2GT_2/A_4), lane 4, the A-stand G-bulge (A_2GA_2/T_4) and lane 5, the perfect duplex (A_4/T_4). The gel was run until the loading dye reached the bottom of the gel (4 hours).

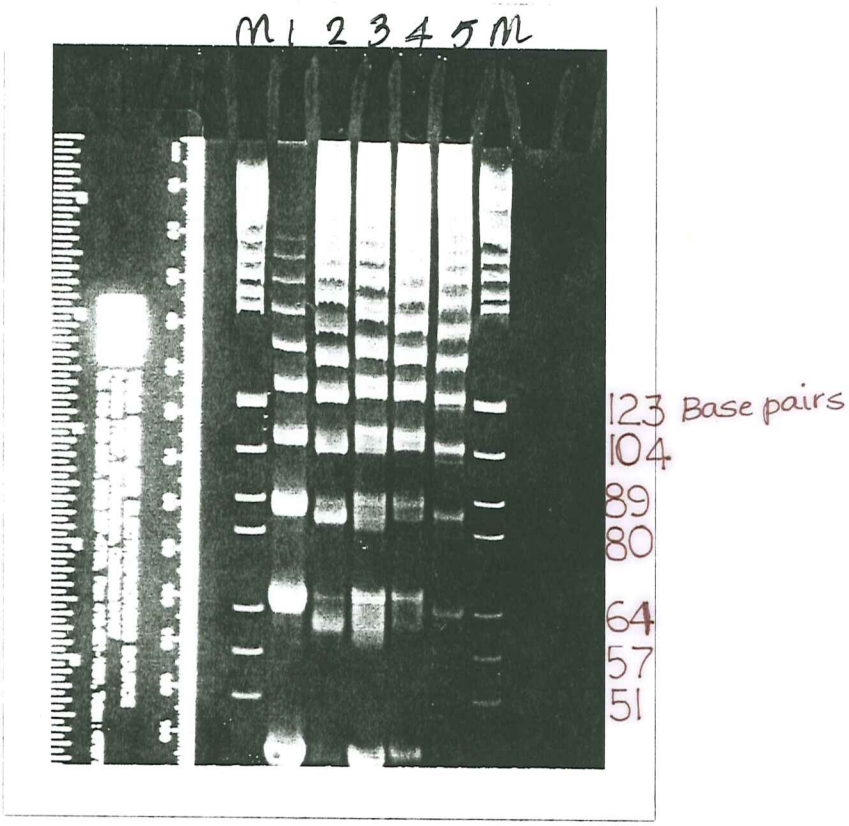

Figure 3: An ethidium bromide stained gel with all the ligated products and the molecular weight marker, pBR322 Hae III digest. Lane 1 contains the interrupted A- and T-tracts (A_2GA_2/T_2CT_2), lane 2, the T-strand C-bulge (T_2CT_2/A_4), lane 3, the T-strand G-bulge (T_2GT_2/A_4), lane 4, the A-stand G-bulge (A_2GA_2/T_4) and lane 5, the perfect duplex (A_4/T_4). This gel was run for 7.5 hours so that the longer DNA sequences would be resolved.

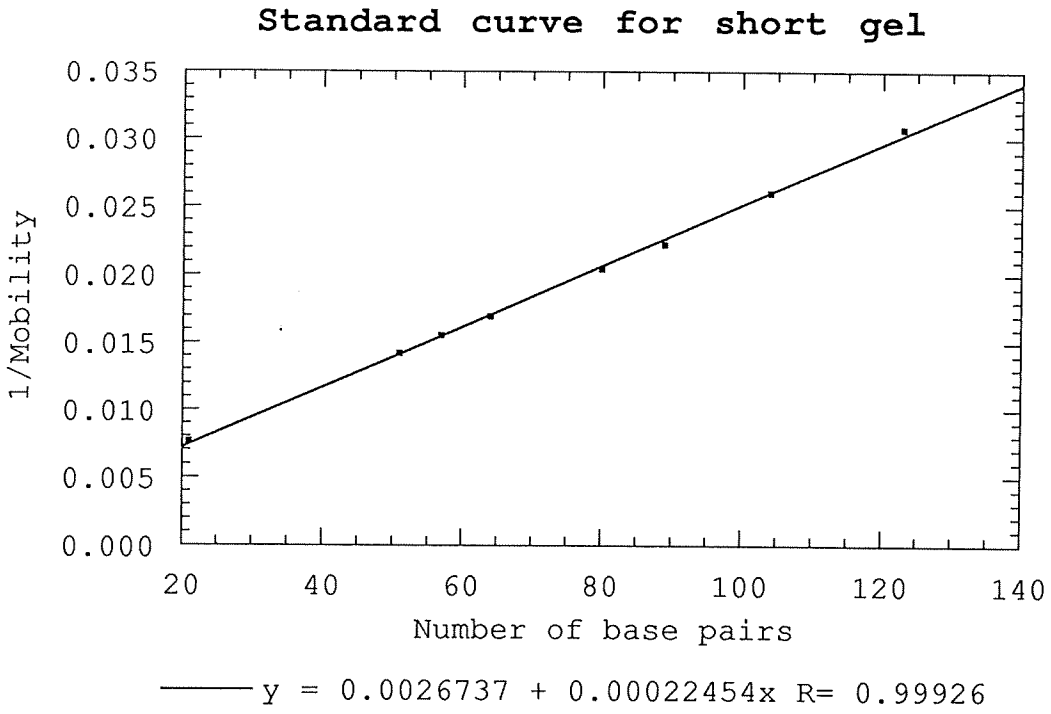

Figure 4: Standard curve calculated from the mobilities of the restriction fragments of a pBR322 Hae III digestion.

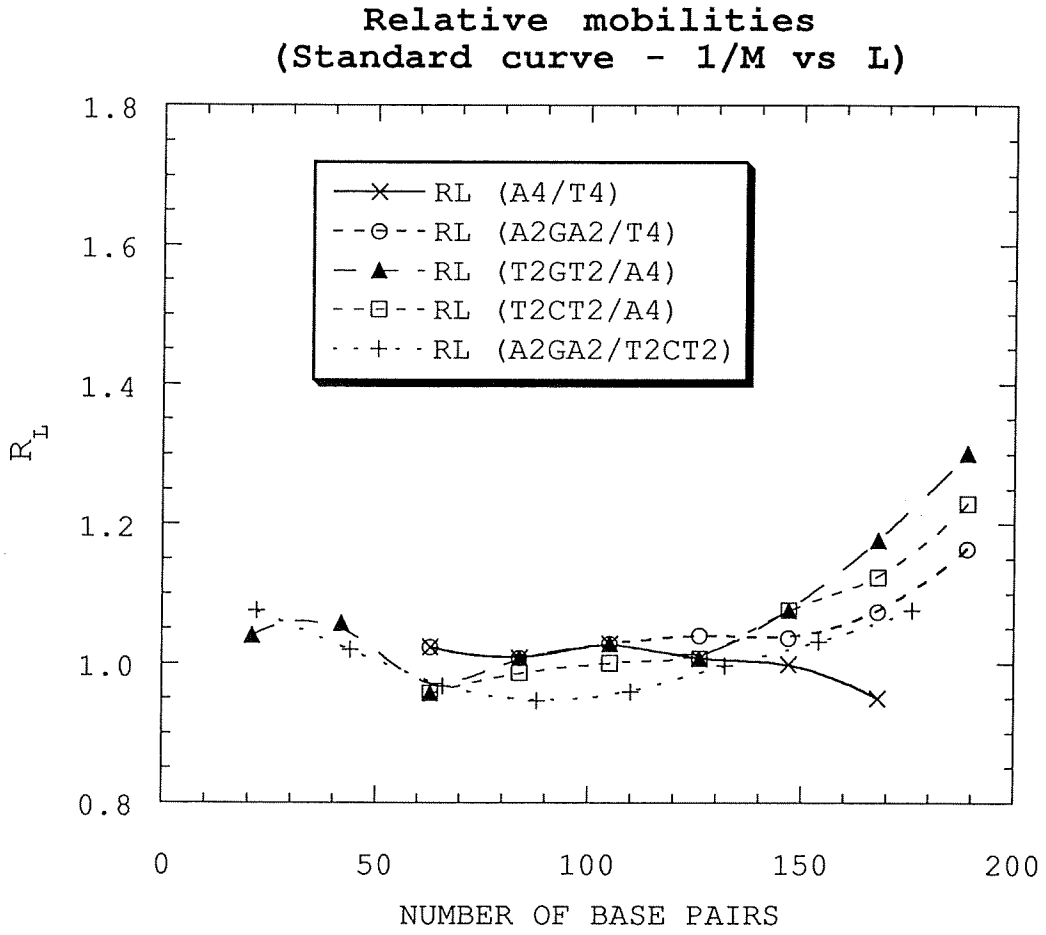

Figure 5: Relative mobilities of oligonucleotides calculated from the sequences seen on the gel in Figure 2 and the Standard Curve in Figure 4.

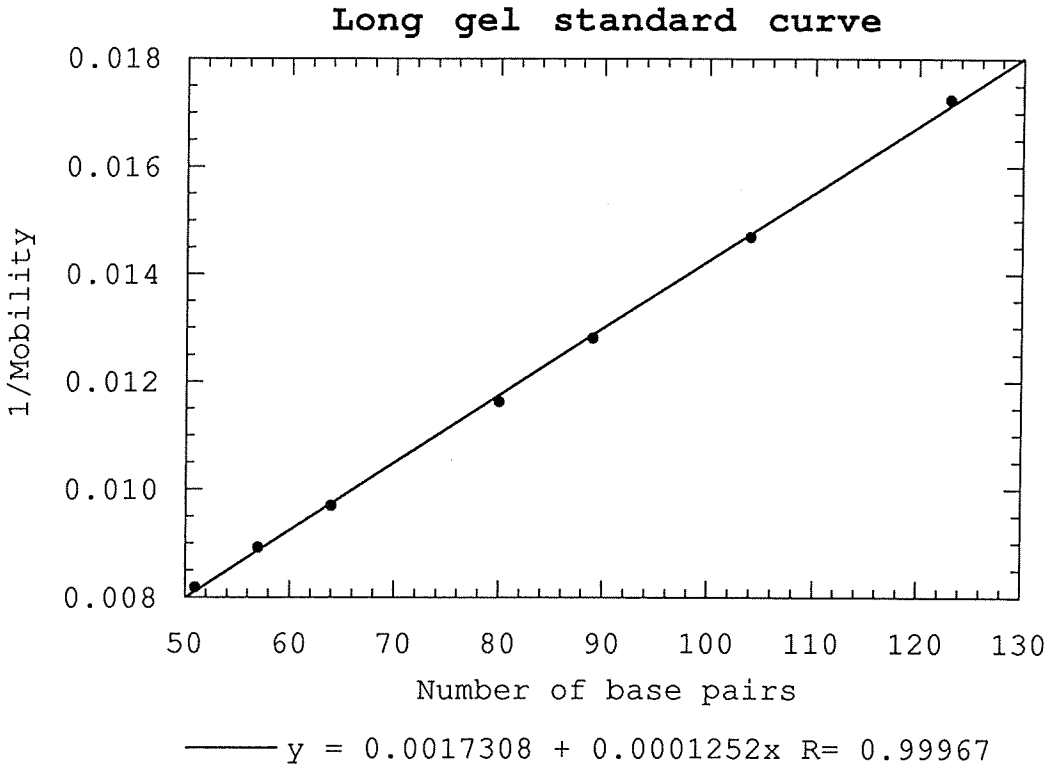

Figure 6: Standard curve calculated from the mobilities of the restriction fragments of a pBR322 Hae III digestion.

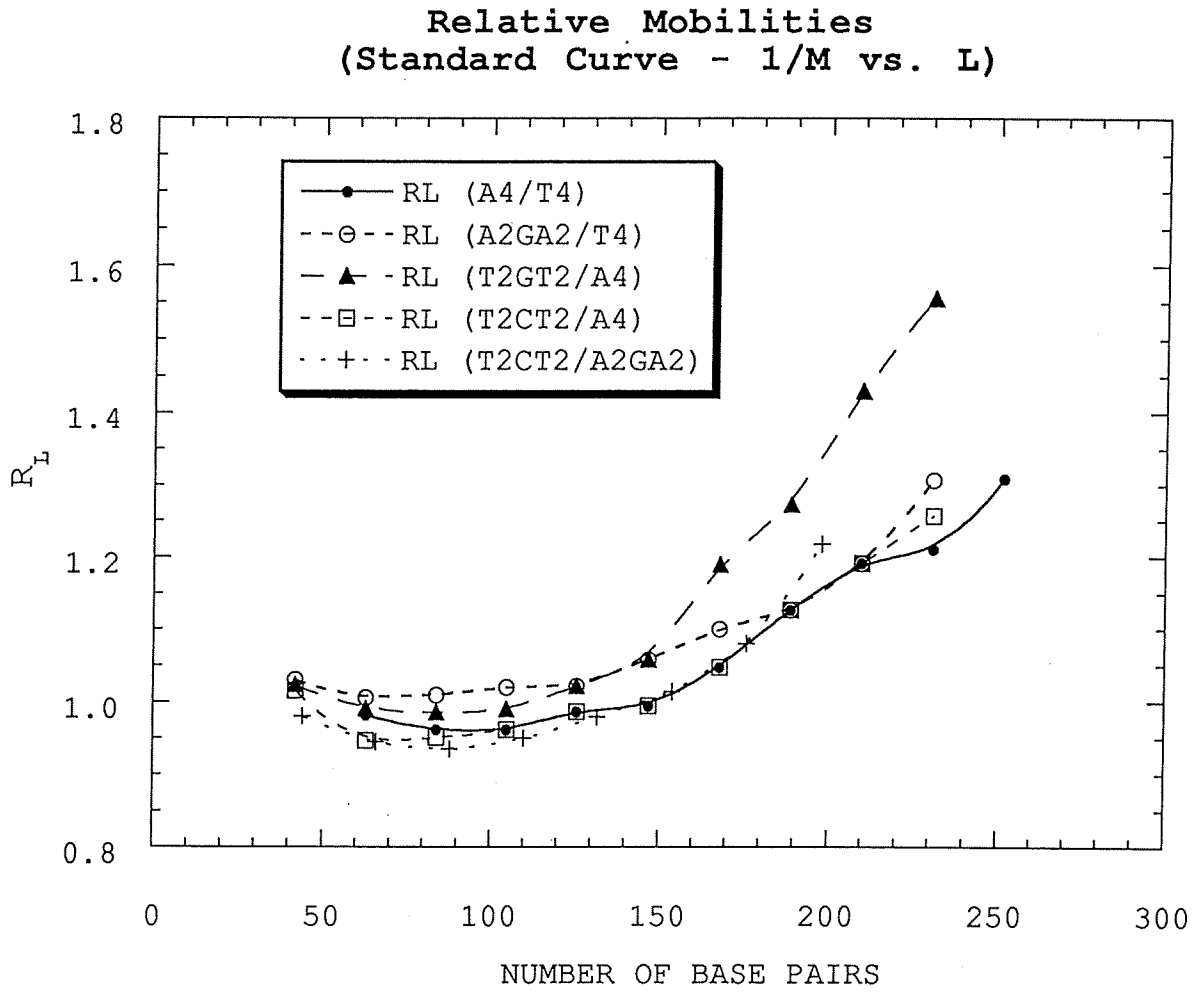

Figure 7: Relative mobilities of oligonucleotides calculated from the sequences on the gel in Figure 3 and the Standard Curve in Figure 6.

Bibliography

1. Abagyan, R.A., Mironov, V.N., Chernov, B. K., Chuprina, V. P. & Ulyanov, A. V. (1990). *Nucleic Acids Res.*, 18(4): 989-92.

2. Brahms, Sabine & Brahms, J. George. (1990). *Nucleic Acids Res.*, 18(6): 1559-64.

3. Chan, Shirley & Breslauer, Kenneth J. (1990). *Biochemistry*, 29: 6161-71.

4. Coll, Miquel, Frederick, Christin A., Wang, Andrew H. J., & Rich, Alexander. (1987). *Proc. Natl. Acad. Sci.*, 84: 8385-89.

5. Diekman, Stephan & McLaughlin, Larry W. (1988). *J. Mol. Biol.*, 202: 823-834.

6. DiGabrielle, Anna D., Sanderson, Mark R. & Steitz,Thomas A. (1989). *Proc. Natl. Acad. Sci.*, 86: 1816-1820.

7. Evans, David H. & Morgan, A. Richard. (1986). *Nucleic Acids Res.*, 14: 4267-80.

8. Gupta, Goutam, Sarma, M. H. & Sarma, R. H. (1988). *Biochemistry*, 27: 7909-7919.

9. Hagerman, Paul J. (1986). *Nature*, 321: 449-450.

10. Hagerman, Paul J. (1990). *Biochemistry*, 29: 1980-83.

11. Hagerman, Paul J. (1990). *Annual Rev. Bch.*, 59: 755-781.

12. Hsieh, Cheng-Hsilin & Griffith, Jack D. (1989). *Proc. Natl. Acad. Sci.*, 86: 4833-37.

13. Hubner, Philipp, Haffter, Pascal, Iida, Shigeru & Arber, Werner. (1989). *J. Mol. Biol.*, 205: 493-500.

14. Kitchin, Peter A., Klein, Viiu A., Ryan, Kathleen A., Gann, Kathryn L., Rauch, Carol A.,Kang, Doe S., Wells, Robert D. & Englund, Paul T. (1986). *J. Biol. Chem.*, 261: 11302-9.

15. Koo, Hyen-Sook & Crothers, Donald M. (1988). *Proc. Natl. Acad. Sci.*, 85: 1763-67.

16. Koo, Hyen-Sook, Dark, Jacqueline, Rice, Janet A. & Crothers, Donald M. (1990). *Biochemistry*, 29(17): 4227-34.

17. Koo, Hyen-Sook, Wu, Hen-Ming & Crothers, Donald M. (1986). *Nature*, 320: 501-506.

18. LeBlanc, Darryl A. & Morden, Kathleen M. (1991). *Biochemistry*, 30: 4042-4047.

19. Losa, Riccardo, Omari, Stefano & Thoma, Fritz. (1990). *Nucleic Acids Res.*, 18(12): 3495-3502.

20. Lozinski, Tomasz, Adrych-Rozek, Katarzyma, Markiewicz, Wojciech & Wierzshowski, Kazimierz L. (1991). *Nucleic Acids Res.*, 19(11): 2947-53.

21. Marini, Joan C., Effron, Phillip N., Goodman, Thomas C., Singleton, Charles K., Wells, Robert D., Wartell, Roger M. & Englund, Paul T. (1984). *J. Biol. Chem.*, 259(14): 8974-8979.

22. Martello, Pamela A., Bruzik, James P., deHaseth, Pieter, Youngquist, R. Scott & Dervan, Peter B. (1989). *Biochemistry*, 28: 4455-61.

23. Morden, Kathleen M., Gunn, Bonnie M. & Maskos, Karol. (1990). *Biochemistry*, 29: 8835-45.

24. Morden, Kathleen M. & Maskos, Karol. (1993). *Biopolymers*, 33: 27-36.

25. Nadeau, James G. & Crothers, Donald M. (1989). *Proc. Natl.Acad. Sci.*, 86: 2622-26.

26. Nelson, Hillary C. M., Finch, John T., Bonaventura, F. Luisi & Klug, Aaron. (1987). *Nature*, 330: 221-26.

27. Rice, Janet A. & Crothers, Donald M. (1989). *Biochemistry*, 28(10): 4512-16.

28. Rickwood, D. & Hames, B. D., Editors. Gel Electrophoresis of Nucleic Acids : A Practical Approach. Washington D. C.: IRL Press, 1982.

29. Sarma, Mukti H., Gupta, Goutam & Sarma, Ramaswamy H. (1988). *Biochemistry*, 27: 3423-34.

30. Searle, Mark S., & Wakelin, Laurence P. G. (1990). *Biochemica et Biopphysica Acta*, 1049: 69- 77.

31. Travers, Andrew A. (1990). *Cell*, 60: 177-180.

32. Trifonov, Ed N. (1991). *TIBS*, 467-470.

33. VanWye, Jeffrey D., Bronson, Edward C. & Anderson, John N. (1991). *Nucleic Acids Res.*, 19(19): 5253-61.

34. Wang, Yuh-Hwa & Griffith, Jack. (1991). *Biochemistry*, 30(5): 1358-63.

35. Wells, Robert D., Collier, David A., Hanvey, Jeffery C., Shimizu, Mitsuhiro & Wohlarb, Franz. (1988). *Faseb Journal*, 2: 2939-49.

36. Yoon, Chun, Prive, Gilbert G., Goodsell, David S. & Dickerson, Richard E. (1988). *Proc. Natl. Acad. Sci.*, 85: 6332-36.