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Tracts of A·T base pairs retard the electrophoretic mobility of short DNA duplexes

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ABSTRACT

An investigation of the mobility of short duplexes of DNA -octamers and decamers-on polyacrylamide gels is presented, showing that molecules containing less than one helical turn exhibit sequence dependent mobilities. Analysis of chains with different sequences indicates that any arrangement of two or more adjacent A.T base pairs causes a duplex to move more slowly than does any combination of isolated A.T pairs This behavior appears to be an intrinsic property of these sequences, since the anomaly persists in the absence of magnesium or presence of spermine and is not due to strand dissociation. In two decamers we studied, the position of A.T tracts within a duplex can be shown to influence mobility: the sequence GA_4T_4C associated with bending or curvature of the helix axis when ligated into polymers migrates more slowly than the corresponding sequence GT_4A_4C, polymers of which migrate as linear B DNA.

INTRODUCTION

Certain sequences can exert a strong effect on the electrophoretic mobility of DNA in polyacrylamide gels. Early observations on the migration of SV40 restriction fragments revealed that particular chains have different mobilities from others of similar sizes (1). However the most striking behavior is seen in the case of DNA isolated from kinetoplasts of the tropical parasite L. tarentolae (2), in which a repeating sequence of adjacent A.T pairs has been associated with a bend in the helix axis (3-5). The phenomenon is attributed to a local structural variation of A_n (n>4) tracts in DNA (5,6): If repeated in phase with the period of the helical repeat in DNA, the helix axis appears to curve, and striking differences are seen in the mobility of double stranded DNA in polyacrylamide gels (4-7). Experiments describing this behavior are carried out on fragments of relatively long chain lengths- 50 b.p. or more. Recent NMR and crystallographic studies are working to demonstrate bending of the helix axis within much shorter duplexes-10

b.p. or so. There is then a question as to whether or not local structural variation can be detected at this level. We show here that high percent polyacrylamide gels can resolve structural differences in duplexes of this size.

In the course of investigating the electrophoretic properties of branched DNA complexes formed from oligonucleotides (8), we noted that certain duplexes corresponding to the arms of a four armed junction migrate differently from others, under conditions that stabilize association to form duplexes - low temperature and high Mg^{2+}. The original set of four octameric duplexes contains three chains in which three A.T pairs adjoin: 8a-c in Figure 1. The fourth octamer, 8d, lacks this property, and migrates more rapidly. Using the set of nine octamer duplexes shown in the table, we can trace this electrophoretic behavior to the presence of a two or more adjacent A.T pairs in the sequences: apparently without regard to their order. The standard conditions we use - 12.5 mM Mg, pH 8, 4 ^{o}C- favor duplex formation even in short chain lengths (9). The role of duplex stability per se has been investigated, as has the effect of gel composition and other counterions. Our conclusion is that this number of A.T pairs is associated with an intrinsic structural difference rather than local electrostatic perturbation or counterion binding. Using a pair of decamers employed in NMR experiments - GA_4T_4C and GT_4A_4C- we find that the former has lower mobility than the latter, showing that position of longer tracts in a duplex can exert an effect on mobility in short duplexes as well as in repetitive longer chains (6).

EXPERIMENTAL

Synthesis of DNA. Individual strands are synthesized on an automated DNA synthesizer (Applied Biosystems 380B) using phosphoramidite techniques (11). Deprotection of bases is accomplished by reaction with 30% ammonium hydroxide overnight at 55 ^{o}C. The strands are dried with a rotary evaporator, coevaporated twice with absolute ethanol, and dissolved in water. The solution is heated to 50 ^{o}C and injected on a Perkin-Elmer HPLC unit equipped with a DuPont Zorbax column. Gradient elution is carried out using two solvents: A, containing 20% acetonitrile: 80% .02 M sodium phosphate (monobasic) and B, containing 1.0 M NaCl dissolved in A. Strands are eluted using a linear gradient of B at a rate of 0,5 %/ minute. Fractions containing the major absorbance peak are precipitated with ethanol (-20 ^{o}C), and dialyzed exhaustively against

Sequence:	Designation:
CGCAATCC GCGTTAGG	8a
CCGAATGC GGCTTACG	8b
GCCATAGT CGGTATCA	8c
TGAGCACG ACTCGTGC	8d
TGGAACCG ACCTTGGC	8e
TGGATCCG ACCTAGGC	8f
TGGTACCG ACCATGGC	8g
TGAGGACG ACTCCTGC	8h
GGCTAACG CCGATTGC	8i
GAAAATTTTC CTTTTAAAAG	10a
GTTTTAAAAC CAAAATTTTG.	10b

Figure 1. Sequences used in the electrophoretic experiments reported here. Note that duplexes 8a, 8b, 8c and 8i contain 3 contiguous AT pairs, while 8e, 8f and 8g are sequences with 2 adjacent AT pairs. AT pairs do not abut each other in 8d or 8h.

distilled water. Concentrations of each strand are estimated from the absorbance at 260 nm of samples at 80 ^{o}C, at which temperature absorbances reflect the sequence of bases present only (12). The sequences of the strands and duplexes used in this study are shown in Fig.1.

Polyacrylamide gel electrophoresis. Slab gels containing a specified percent composition in acrylamide are made up to 5% bisacrylamide/acrylamide, and run in a running buffer containing 40 mM Tris-HCl, 20 mM acetic acid, 2mM disodium EDTA- TAE buffer, with magnesium acetate unless otherwise specified. The loading buffer consists of equal volumes of TAEMg and glycerol, with 0.02 % Bromophenol Blue and Xylene Cyanol FF tracking dyes. Gels are stained in a solution of 1:1 formamide: water containing .01 % Stainsall dye (Eastman Kodak). Experiments are run on Hoefer 600 units at 10 V/cm, with temperature regulation from a circulating water bath, usually at 2

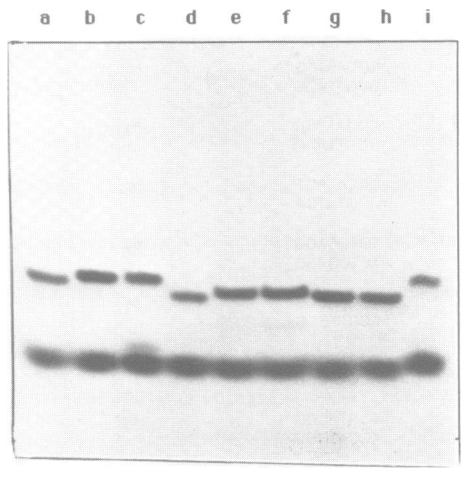

Figure 2. Electrophoretic mobility of the nine complementary duplexes in a 20% polyacrylamide gel, in TAE buffer with 12.5 mM Mg^{2+}. Lanes a to i correspond to each of the octamer sequences 8a-8i designated in Figure 1. The lower band in each lane is that of the tracking dye, Bromophenol Blue.

°C, giving a running temperature of 4 °C. Gels are scanned with a Hoefer densitometer in reflectance mode, interfaced to a PC.

RESULTS

Sequence dependence of oligomer mobility.

The nine non-self-complementary duplexes shown in Fig 1 have been subjected to electrophoresis on polyacrylamide as described, using a gel of 20% acrylamide, 12.5 mM Mg acetate and at 4 °C. Fig. 2 shows the effect: duplexes 8a, 8b, 8c and 8i migrate more slowly than the others. Duplexes containing pairs of adjacent A·T pairs-8e, 8f, and 8g- appear to be faster than the former, and slower than 8d and 8h. This experiment suggests that the mobility of octamers depends on the presence of adjacent A·T base pairs in any sequential order- for example AA, or AT. This is in contrast to the observations of apparent tendency to "bend" the helical axis, which requires the specific arrangement AAA, A_4, etc. (3, and see below). To characterize the phenomenon further, we have tested whether or not these differences in mobility reflect differences in the stability of the duplexes, leading to a process of strand dissociation during migration, or differential binding of counterions by different sequences. Running gels at different temperatures provides the most

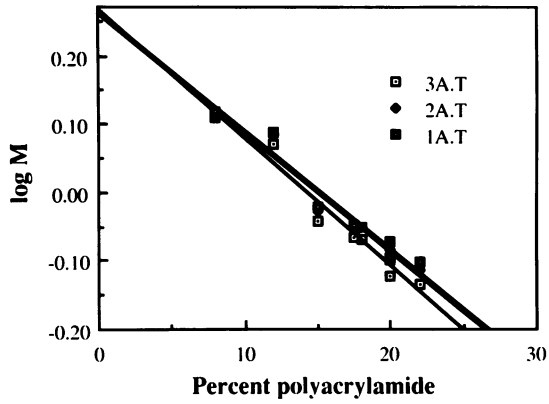

Figure 3. Dependence of mobility on gel composition. Superimposed Ferguson plots of three octamer duplexes are shown the sequences of which contain isolated AT base pairs (1A·T) ,two adjacent AT base pairs (2A·T) and three adjacent AT base pairs (3A·T).

direct test of the former hypothesis: if the phenomenon is due to strand dissociation, increasing temperature should enhance differences in apparent mobility. Heating to 16 ^{o}C does not alter the differential mobilities of the duplexes, leading us to conclude that neither strand dissociation nor "fraying" is responsible for the behavior. This is supported by the counterion experiments described below.

Effect of Gel Concentration.

The electrophoretic migration of oligonucleotides is sensitive to the concentration of acrylamide in the gel (12). The dependence of mobility on acrylamide concentration -called a Ferguson plot (13,14)- provides a means of comparing the apparent frictional coefficients of different molecules. We have reported for example that four- armed DNA junctions migrate with distinctive mobilities and dependence on acrylamide concentration that differ from those of standard duplexes (8). Fig. 3 illustrates the dependence of the mobility of duplexes containing one, two and three adjacent A.T base pairs on gel concentration. While there is some scatter in the measurement due to variation in the band profiles, the Ferguson plots suggest that the slopes are different within experimental error, and that differences in mobility extrapolate to disappear at 0% acrylamide. This intercept measures the free mobility of of a chain, determined by charge density independently of frictional behavior (13) , and in principle can distinguish a structural from an electrostatic basis for the differences in mobility.

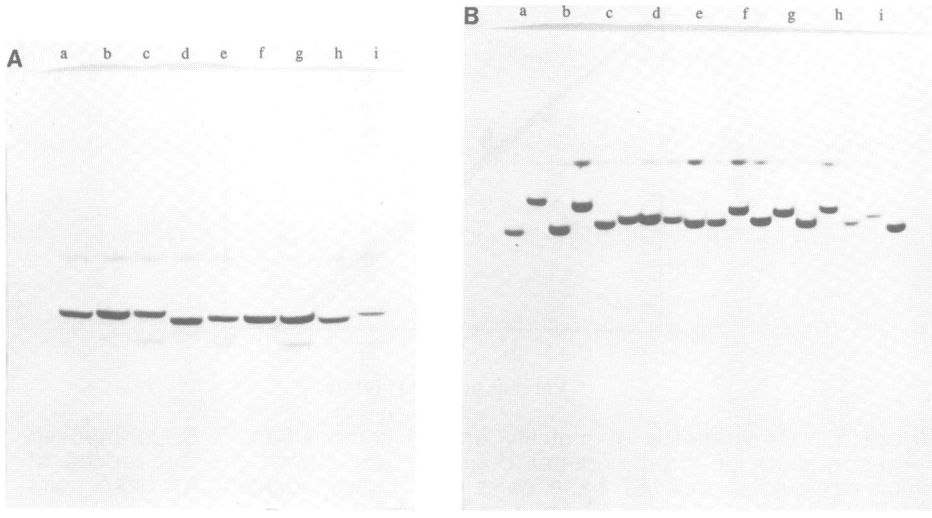

Figure 4. A. Electrophoretic mobility of nine complementary duplexes in a 20% polyacrylamide gel, in TAE buffer with 10mM NH_4^+. Lanes a to i correspond to each of the octamer sequences 8a-8i in Fig. 1. The upper band in each lane is that of the tracking dye, Bromophenol Blue. B. Mobility of the eighteen individual strands in the nine complementary duplexes in Fig.1, in TAE buffer. Each set of two strands corresponds to those in one octamer, 8a-8i, designated in Fig.1.

Effect of Counterion.

If Mg^{2+} is replaced by 10mM ammonium , the duplexes maintain their relative order of mobilities (Fig 4A). In TAE buffer alone, the same relative order can be seen, although some of the duplexes dissociate. At lower Mg concentration-1.25 mM, the order is also retained- consistent with the idea that the mobility is an intrinsic property of a duplex, and not of the strand dissociation reaction. In fact, the mobilities of the pairs of individual strands that make up each octamer do not show any correlation with mobilities of the duplexes. This is shown in Fig 4B which presents nine sets of single stranded octamers run in TAE with no Mg. There are differences between the mobilities of different pairs, but none that correlate with the mobilities of the duplexes. For example, the single strands of octamers a and b have different mobilities, while those of octamers c and i do not. These four duplexes are equally retarded in the duplex form. To decide whether differences in mobility might reflect differential mono- or divalent ion binding among the duplexes, we determined the mobilities in the presence of the organic polyamine

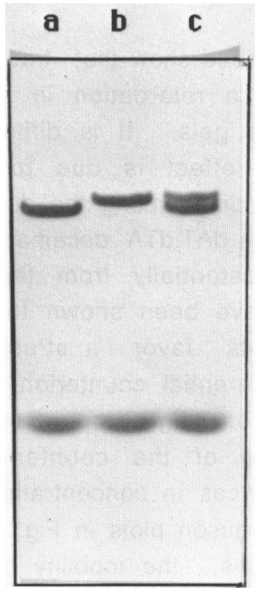

Figure 5. Electrophoretic mobility of two decamer duplexes (10a and 10b in Fig. 1) in a 28 % polyacrylamide gel. Lanes a and b contain decamers GT_4A_4C and GA_4T_4C respectively, and lane c contains a mixture of both duplexes, showing that they resolve under these conditions.

spermine, which has a charge of +4. In the presence of 0.1 mM spermine, there is no difference in the relative values from that seen with Mg^{2+} or in the presence of NH_4+.

Effect of Sequence.

Mobility differences among the four octamers containing tracts of three A.T pairs are hard to distinguish, even in gels with a high percentage of polyacrylamide. However, a strong effect of position of the A.T tract within a decamer is demonstrated in Fig. 5, which contrasts the mobilities in a 28% polyacrylamide gel of two self-complementary decameric duplexes containing an identical tract of four A.T pairs in different positions within the sequence: entries 10a and 10b in Fig. 1. Extended electrophoresis in gels of high composition might thus be expected to reveal differences in the octamers with tracts of three A.T's in different arrangements. However, even electrophoresis at 32% polyacrylamide concentrations shows no separations among the octamers of this study which contain 3 contiguous A-T pairs.

DISCUSSION.

The experiments described show that tracts of two or more A.T base pairs are associated with a retardation in the mobility of short DNA duplexes in polyacrylamide gels. It is difficult to distinguish between the possibilities that the effect is due to structural differences or differences in counterion binding among the duplexes, or a combination of both. A 2D NMR study of a dAT.dTA decamer suggests that the structure of this duplex differs substantially from that of B DNA (15), while sequences of dAA. dTT have been shown to be structurally anomalous (16,17). Two arguments favor a structural basis for the mobility behavior rather than a differential counterion binding effect. The first is that the relative order of migration of the octamer duplexes is unaffected by the charge of the counterion used-NH_4^+, Mg^{2+}, or spermine^{4+}- or the differences in concentration we have examined. The second is based on the Ferguson plots in Fig. 3. From both experimental and theoretical considerations, the mobility of charged particles of any shape is related to the concentration of the sieving gel by a semi-logarithmic relation:

$$\log M = \log M_0 - K_R \cdot [T]$$

where M is the mobility in a gel of acrylamide concentration [T], M_0 is the free mobility, at [T] = 0, and K_R - the slope of the Ferguson plot-is the retardation coefficient (13). Linear Ferguson plots have been reported for both RNA and proteins in SDS (14). The free mobility M_0 is a measure of the charge density of the migrating species, whereas K_R reflects the free volume-size and shape-of the particle as it moves through the sieving medium. The dependence of the mean slopes and intercepts of the least squares fits to the Ferguson plots for all the octamers studied on A.T tract length is the following:

AT Tract length:	log M_0	K_R (% $^{-1}$)
1	.265 +/-.002	.0173+/-.00005
2	.263 +/-.002	.0175+/-.0001
3	.263 +/-.002	.0184+/-.0003

There is no trend in the free mobilities, while K_R increases with the tract length. This is consistent with a change in shape between duplexes with one, two or three adjacent A·T pairs, rather than a change in charge density.

The mobilities of the four duplexes containing tracts of three adjacent A.T pairs are similar, and we have not succeeded in resolving them from each other even in 30% polyacrylamide gels. However, a difference in the mobilities of two decamers containing identical tracts of AAAA/TTTT can readily be demonstrated by using high percentage acrylamide gels to take advantage of the anticipated effect on K_R, as seen in Fig.5. These two sequences exhibit large scale differential mobilities when ligated to form polymers containing five or more repeats (5,6). We see here that a difference can be detected in monomeric decamer duplexes, providing a basis for high resolution studies of chains consisting of one turn of helix or less. It is not possible to determine from this gel whether the mobility differences reflect structural or (anisotropic) flexural differences between the two duplexes. Since we find no evidence of an effect of position or arrangement among the adjacent A·T pairs in the octamer duplexes it is not clear that the effect in the decamers is similar, or even related. Further experiments are required to settle this. We believe that extension of electrophoretic measurements to short duplexes as described here offers a convenient and sensitive method for establishing physical differences between chains of different sequence.

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