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A GEL-CONCENTRATION-INDEPENDENT RETARDATION DETECTED IN TWO FRAGMENTS OF THE *rrn*B P1 PROMOTER OF E. COLI USING TRANSVERSE POLYACRYLAMIDE PORE GRADIENT GEL ELECTROPHORESIS

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SUMMARY: Two fragments of the E. coli *rrn*B ribosomal RNA P1 promoter Upstream Activation Region exhibit a constant gel retardation, over a polyacrylamide gel concentration range of 3% to 10%. Gel retardation is usually seen to increase with polyacrylamide gel concentration as in the case of the 219 base pair Crithidia fasciculata kinetoplast DNA fragment which represents the classic case of sequence directed curvature. Computer modeling of the Upstream Activation Region fragments suggests that their unusual electrophoretic behavior can be accounted for on the basis of a "screw" model proposed by Drak and Crothers (1991, Proc. Natl. Acad. Sci. USA 88,3074-3078).

The conformation of DNA has been shown to depend upon its nucleotide sequence. For reviews see Hagerman (2) and Trifonov (3). Poly-A tracts phased to coincide with the helical repeat of the DNA molecule appear to cause substantial bending of DNA (4,5), although sequences lacking A-tracts may also be bent (6). The best example of the phenomenon of sequence-directed bending is a 219 base pair DNA fragment excised from the minicircles of the kinetoplast of the trypanosome Crithidia fasciculata (7). The sequence of the kinetoplast DNA features the phased A-tracts which are the classic bending motif. The bending found in the kinetoplast fragment may be a way of flagging minicircles as newly replicated or unreplicated during minicircle duplication (8), however a more general role for bent DNA lies in the regulation of gene expression. Sequence-directed DNA bending has been detected in many stretches of DNA in the vicinity of promoter regions (9,10), origins of replication (11,12,13), and in an important control region in the adenovirus genome (14). Bends in DNA per se have profound effects on the expression of some genes (15,16) regardless of the sequence producing the bend.

Abbreviations: UAR = Upstream Activation Region; RL = ratio of apparent length, based on electrophoretic mobility, to the actual length of a DNA fragment ("relative length"); %T = weight percent of acrylamide monomer; TBE = Tris Borate-EDTA buffer (89 mM tris, 89 mM boric acid, 2 mM EDTA); E. coli = Escherichia coli.

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DNA molecules which are bent show reduced mobility during polyacrylamide gel electrophoresis. This effect is often expressed as an RL (relative length) value (17,18). RL is 1.0 for unbent DNA fragments and greater than 1.0 for bent fragments. Most DNAs with documented bends show an increase in RL with increasing gel concentration. Unusual bent DNA molecules showing decreasing or invariant RLs rather than increasing RLs with increasing gel concentration have now been reported (10,19). The difference in the response of these molecules to gel concentration changes is likely to be due to qualitative differences in their shapes.

Transverse pore gradient polyacrylamide gels, with a gradient in gel concentration oriented orthogonally to the direction of electrophoresis, have been effective in distinguishing between conformationally distinct "lariat" and linear RNAs (20). Zacharias et. al. (21) have demonstrated that an anomalously migrating DNA molecule, the UAR from the E. coli *rrn*B P1 promoter, could be detected using a transverse pore gradient polyacrylamide gel. The UAR sequence has been shown to be responsible for a 15-fold enhancement in the transcription of the *rrn*B operon of E. coli (10). Although the UAR sequence lacks the conspicuous A-tracts of the kinetoplast DNA the efficacy of the UAR in enhancing transcription has been shown to be dependent upon the number of helical turns of DNA separating it from the RNA polymerase binding site of the operon (22), supporting the hypothesis that its effects are exerted via a specific, directional bend. Here an instance is reported in which two portions of the UAR (10) exhibit uniform RLs over a wide range of gel concentrations in contrast to the normal behavior of the bent kinetoplast sequence. Computer modeling of these sequences suggests a reason for the difference.

MATERIALS AND METHODS

Transverse Pore Gradient Gels. Transverse pore gradient gels were purchased from Jule Biotechnology, New Haven, Connecticut. The linear gradient in monomer concentration ranged from 3% to 10% while the bis acrylamide crosslinker concentration was held constant at 3%. Gels were run at either 25° C or 4° C in 0.5X TBE using a voltage gradient of 10 V/cm.

Bent DNA Fragments. All restriction enzymes were purchased from Bethesda Research Laboratories, Gaithersburg, Maryland. E. coli strains RLG1510 and RLG1509 were the gifts of Richard L. Gourse, of the Department of Microbiology, University of Wisconsin. DNA fragments UAR135 and UAR165 were cut from the vector psL6 isolated from E. coli strains RLG1510 and RLG1509 respectively using EcoRI and BamHI. The vector pPK201/CAT was isolated from E. coli which were the gift of P. T. Englund of Johns Hopkins University. The 219 base pair kinetoplast DNA fragment was excised from pPK201/CAT using BamHI.

Electrophoresis and Data Acquisition. The kinetoplast and UAR fragments were electrophoresed against the 123 base pair ladder of Bethesda Research Laboratories. The 123 base pair ladder has been used previously as a standard linear ladder for the quantification of migration anomalies in bent DNA fragments (19). Standard plots of LOG(BasePairs) vs migration distance were linear for this standard ladder over the gel concentration range of 3 %T to 10 %T (data not shown). Gels were stained in 0.5μg/ml ethidium bromide and photographed during ultra violet illumination using an 8 bit PC-based video acquisition system (BioPhotonics, Ann Arbor, Michigan). The convex lines representing DNA migration distances across the transverse pore gradient gels (Ferguson curves) were traced by computer as described previously (23).

Data Analysis and Figures. Second order polynomials of best fit were calculated for the traces of the linear size standards and these polynomials were used to generate standard plots of LOG(Base Pairs) vs gel concentration for the size standards at 100 evenly spaced gel concentrations between 3 %T and 10 %T. The apparent lengths for the UAR sequences and the kinetoplast sequence were read from these plots to produce Figure 2. The tertiary structures for the UAR sequences and the kinetoplast sequence were generated using a program for the IBM PC available from the author. The 10 dinucleotide wedge angles used were those calculated by Bolshoy et. al. (6) while the dinucleotide twist angles were those of Kabsch et. al. (24). The wedge angles of Bolshoy et. al. allow the computation of DNA tertiary structure in the absence of phased A-tracts and can accurately predict non-A-tract curvature as well as A-tract curvature (6).

RESULTS

Figure 1 shows the gel patterns obtained for the 219 base pair kinetoplast DNA fragment and the two portions of the UAR sequence at 25° C. The trajectory of the kinetoplast curve in Figure 1A is radically different from those of nearby standards. The lines representing UAR135 and UAR165, seen in the gel in Figure 1B, have trajectories parallel to those of nearby standards. The electrophoresis of UAR135 at 4° C produces a trace which is parallel to that produced at 25° C but is displaced upwards towards the sample well (gel photo not shown). The RL ratios obtained from the transverse gradient gel patterns are plotted for the kinetoplast and UAR fragments in Figure 2. The dramatic linear increase in RL with increasing gel concentration seen for the kinetoplast fragment represents the usual behavior for a bent DNA molecule. The invariance of RL with gel concentration seen for the UAR fragments, both at 25° C and 4° C, is a departure from the expected behavior for bent DNA molecules. The RL ratios for the UAR sequences are about 1.08 at 25° C and 1.90 to 2.0 at 4° C, while RL for the kinetoplast varies from about 3.6 at 3 %T to 5.8 at 9 %T. Using the dinucleotide wedge angles of Bolshoy et. al. (6) and the twist

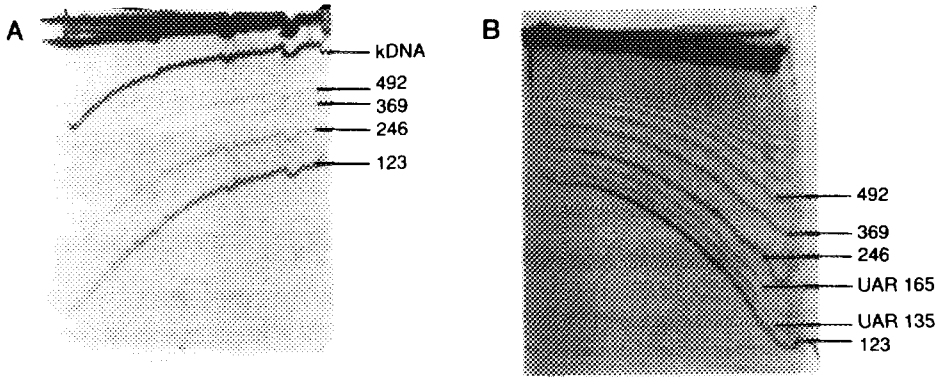

Figure 1. Photographs of two transverse pore gradient gels run at 25° C are shown stained with ethidium bromide. In each case the polyacrylamide monomer concentration varies from 3% to 10% with a crosslinker concentration of 3%. (A) Gel pattern for the 219 base pair kinetoplast DNA fragment. The kinetoplast fragment is marked "kDNA". Other numbers refer to the lengths of size standards. (B) Gel patterns for UAR135 and UAR165.

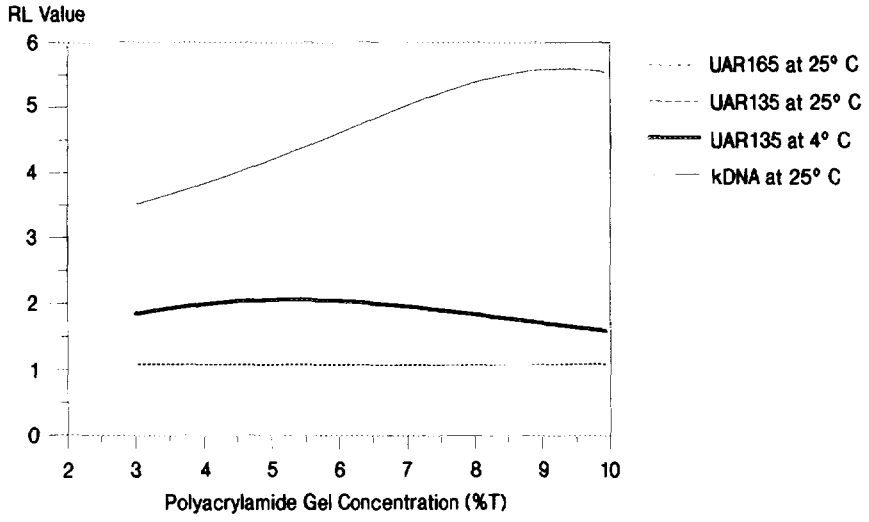

Figure 2. The relationship between RL and polyacrylamide gel concentration is shown for the 219 base pair kinetoplast DNA fragment, UAR135, and UAR165. RLs were measured relative to the DNA fragments of the 123 base pair ladder.

angles of Kabsch et. al. (24), it is possible to compute tertiary structures for the kinetoplast and UAR sequences which are given in Figure 3. The kinetoplast sequence forms a planar circle. The UAR sequences show a gentle superhelical twist which is right handed.

DISCUSSION

The reason that bent DNA molecules migrate slowly in polyacrylamide gels is under investigation. An examination of the variable behavior of the two DNA molecules studied here on transverse pore gradient gels can shed some light on the mechanisms of gel retardation. It is assumed in the following discussion that the radical change in RL observed in the case of the kinetoplast DNA fragment is the result of the molecular shape and not the result of chemical interactions between the DNA and the gel. The most obvious conformational feature of the kinetoplast fragment is the planar, circular curvature seen in the computer predicted structure in Figure 3. This curvature can be hypothesized to be fairly rigid since similar structures are observed using scanning electron microscopy (25) and the length of 219 base pairs is close to the dynamic persistence length for DNA of 200 base pairs (26). Covalently closed circular DNA of greater than about 500 base pairs cannot enter a polyacrylamide gel of concentration 5 %T (27), probably due to the inability of these DNAs to move through the gel end-on. Kinetoplast DNA also has this problem if its curvature is taken a rigid; it can enter the gel but because of its circular form it finds itself progressively hindered at higher gel concentrations and is unable to become oriented in such a way as to move through the gel end-on. The two UAR fragments studied here are also bent to the extent that they are mildly retarded in their migration. It is

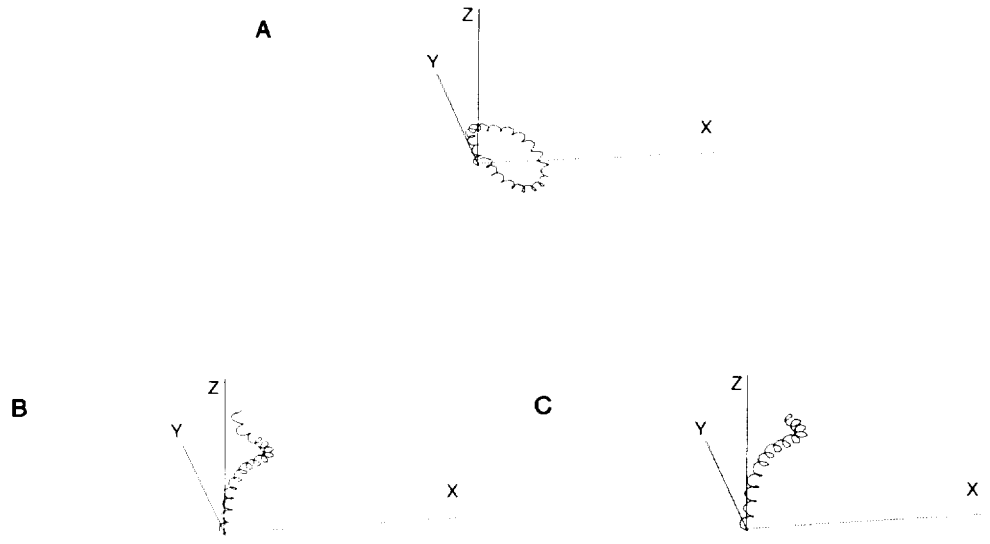

Figure 3. Tertiary structures calculated for the three DNA fragments studied are shown. The structures were calculated using the dinucleotide roll and tilt angles of McNamara et al. (17) and the dinucleotide twist angles of Kabsch et al. (24). One strand of the DNA double helix is shown to allow comparison of its right handed twist with that of the superstructure of the entire molecule. The figures show two dimensional projections of three dimensional structures. The X, Y, and Z axes of the coordinate system are illustrated. (A) Predicted structure for the 219 base pair kinetoplast DNA fragment. (B, C) Predicted structures for UAR135 and UAR165, respectively.

reasonable to assume that, as in the case of the kinetoplast DNA, the bends seen in Figure 3 are rigid. The overall shape of the UAR fragments is linear and these fragments can, therefore, migrate end-on but with some restrictions on their movement. A mild degree of retardation, then, is not unexpected. One would predict the effects of the constraints on movement imposed by the bends to become more pronounced at higher gel concentrations as in the case of the kinetoplast DNA. This is not reflected in the RLs for these two fragments, however, since the RLs are invariant with gel concentration (Figure 2). A compensatory mechanism which accelerates the UAR sequences in their progress through the gel as gel concentration increases could offset an increase in RL due to the constraints on movement imposed by the bends. Such a mechanism has been proposed by Drak et. al. (1) who have suggested that in cases where a DNA molecule exhibits a superhelical twist, this twist may hinder or improve migration through the gel depending on whether it has the same handedness as that of the DNA double helix. If the superhelical twist has the same handedness as the twist of the DNA double helix, then the two twists are "coupled" and migration through the gel is enhanced. In the case where the two twists are of opposite handedness, the twists are said to be "uncoupled" and migration through the gel is hindered. The migration mechanism implied here is that of a DNA molecule penetrating the gel

matrix using a screw-like rotational motion. Figure 3 shows that both of the UAR sequences show a shallow right handed superhelical twist which would show a "coupling" with the right handed twist of the DNA helix. A screw-type migrational mechanism would be expected to exert a more pronounced effect as gel concentration increases due to increased contact between the DNA "screw threads" and the gel. Hence, an increasing retardation in migration speed due to bend-induced restraints on movement may be balanced by an increasing acceleration due to coupled screw movements. The result of these two opposing forces could produce a gel retardation which is independent of gel concentration. It should be noted that the data of Zacharias et. al. (21) for a larger 267 base pair DNA fragment containing the UAR sequences studied here show an increase in gel retardation with increasing gel concentration. It is possible that the additional 100 base pairs in this fragment accentuate the effects of the bends by placing the "screw" structure of the UAR sequence in the center of a larger molecule. Central bends give rise to stronger electrophoretic retardations (2) which might overcome the compensatory "screw" acceleration suggested above.

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