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On the mobility behavior of a curved DNA fragment located in circular permutation†

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Abstract Experimental and theoretical investigations on the mobility behavior of a set of permuted fragments with a K-DNA insert is reported. The fragments with the permuted flanking sequences have the K-DNA insert located differentially with respect to the fragment ends. The fragment wherein the insert is located in the center showed maximum retardation as compared to fragments where the insert was at the end. The experimental analysis is also in accord with the theoretical investigation.

Key words: Intrinsic curvature; Cyclically permuted; Cyclically located

1. Introduction

The functional role of sequence-dependent DNA curvature in replication, transcriptional regulation and chromatin structure has been found in a number of different biological systems [1]. Since biological events are regulated at the molecular level by site-specific association between specialized proteins and DNA (e.g. *Lac* and *trp* operon regulation; see reviews [2–4] and references therein), it is believed that sequence-specific protein–DNA recognition and the ability of certain proteins to compete for multiple binding sites is regulated at several levels by local structure and conformation of the binding partners. Some of the defined protein structural elements that bind to specific DNA sequences include helix–turn–helix motif found in eukaryotic regulatory proteins; zinc finger motif found in DNA and RNA binding proteins; α structure found in *Eco*RI; and antiparallel β-strands as in the *E. coli met* repressor. A number of specific systems corroborating this have been extensively reviewed (see [2–4]).

The observation that certain dinucleotides in chromatin DNA sequences in general, and AA and TT in particular, tend to occur at multiple distances of the DNA helical repeat was made some years ago [5]. DNA was also thought to possess a sequence-dependent intrinsic curvature and anisotropic bendability [6]. The sequence-dependent curvature has since been subjected to intense theoretical and experimental investigation and is now a well established fact [7–10].

Essentially two classes models that explain the superstructural features of DNA which have stood the test of time are: the nearest neighbor wedge model [6,10]; and the junction bending model [11,12]. The wedge model was originally proposed by Trifonov [6], and is based on the assumption that different stacks of neighboring base pairs have different dihedral angles between them, as if some small sequence-dependent wedge were inserted between them. The axial deflection of the successive wedges combine to form a planar curve and is particularly significant for the AA sequence. The junction model assumes that AA tracts containing more than three adenines would adopt a helical conformation different from the canonical B structure. The net curvature in this case is caused by the axial deflection that arises from the structural discontinuities at the junction between locally some what different sequence dependent forms of DNA, like inclination of DNA axis at the junction between B-form and A-form [13]. The latter having base pairs appreciably tilted towards the axis. The junction bend model has been explained in terms of the wedge model. In both models the phasing of the $[A]_n$ tract plays an important role and the curvature of individual bending elements add coherently to produce a larger overall bend. The wedge presentation is able to provide a general description for any succession of stacked elements irrespective of the formation of any local unusual DNA structure, or whether the wedge elements are constant or variable. Such an equivalence has been demonstrated in the case of constant wedges [14]. The recent experimental estimation of wedge angle values for the 16 possible dinucleotide stacks in DNA [15], which is a complete formulation of the wedge model, describes the contribution made to DNA curvature by all dinucleotide steps along the double helix.

We report here the mobility behavior of a set of permutated fragments wherein the insert (which is intrinsically curved) per se is not permuted while only the flanking sequences are permuted and the insert only slides from one end of the fragment to the other.

2. Materials and methods

Agarose, acrylamide, Bis-acrylamide, TEMED, APS, Tris-boric acid, EDTA and $MgCl_2$ were purchased from Sigma Chemical Co Ltd. Restriction enzymes were from Pharmacia, Boerhinger Mannheim or New England Biolabs; Klenow fragment of DNA polymerase, T4 DNA polymerase and T4 DNA ligase were from Pharmacia. The enzymes were used according to the suppliers specifications. The plasmid pBend2 was a generous gift from Sankar Adhya [16].

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†This paper is dedicated to the memory of Prof. John Barnabas (1927–1994).

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2.1. DNA manipulation

The minicircle fragment comprising the universal minicircle sequence were cloned into pBend2. The *Eco*RI–*Pst*I ends of fragment E3B from the minicircle pLdKE3 (from 1–222 bp, EMBL access no. X68026) were repaired with T4 DNA polymerase in the presence of dNTPs and cloned into filled *Xba*I ends of pBend2 to obtain pMMN32.

2.2. Analysis of the permuted fragments

Cyclic permutation of the DNA sequence makes it possible to study the effect of relative location of the wedge sequence, while keeping the total length of the molecule constant. A set of such molecules were obtained by digesting the recombinant plasmid pMMN32 using a suitable set of enzymes. These DNA fragments were analyzed on a non-denaturing 12% polyacrylamide gel in 1 × Tris-borate-ethylenediaminetetraacetic acid buffer (pH 8.3) at constant voltage of 4–5 V/cm at 4°C. The gels were stained with ethidium bromide and then photographed.

2.3. Trajectory plots and curvature maps of permuted fragments

The plots of Figs. 4 and 5 were calculated using the curvature programme [17] based upon the 16 DNA wedge angles which have been determined experimentally [15]. These generated trajectories provide both the degree of curvature and its orientation.

3. Results and discussion

We present here the results of our experiments on the mobility behavior of a set of constant length permuted fragments with a K-DNA (E3B) insert. The nucleotide sequence of the minicircle pLdKE3 (EMBL access. no. X68027) revealed the presence of several 'A' tracts surrounding the universal minicircle sequence (UMS), GGGGTTGGTGTAA. The minicircle was analyzed for regions with high curvature using the CURVATURE programme [17]. Fig. 1 shows the curvature expressed in DNA curvature units [18] of the entire minicircle. The regions with high curvature values correspond to regions which are maximally curved. Hence this region, E3B (*Eco*RI–*Pst*I; 1–222 bp) containing the UMS were subcloned into pGEM4Z, for further analysis. The E3B fragment moved anomalously in polyacrylamide gel at room and at lower temperature. The mobility of the E3B fragment was studied under different conditions and it was found to be retarded in every case (data not shown).

We were interested to learn about the mobility behavior of a curved DNA fragment when it gets differentially located on a straight piece of DNA fragment. In order to study this, the subfragment E3B was cloned into pBend2, a vector which contains two identical DNA segments containing 17 restriction

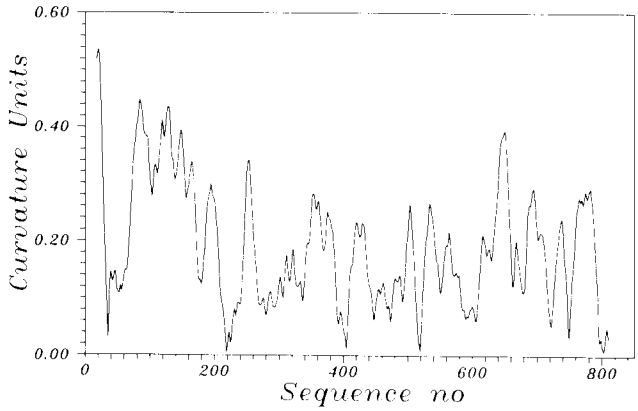

Fig. 1. Curvature profile of the entire minicircle pLdKE3 expressed in DNA curvature units, which is the average curvature of DNA in the nucleosome core particle,1/42.8 A (see [18]).

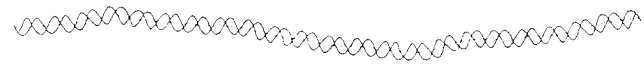

Fig. 2. DNA path of the pBend2 fragment without the K-DNA insert.

sites in a direct repeat spanning a central region containing cloning sites. The plasmid can generate a large number of DNA fragments of identical length in which the K-DNA fragment is located in circular permutation.

The analysis of the pBend2 fragment revealed that it had no intrinsic curvature associated with it. This is clearly evident from Fig. 2 which depicts the DNA path evaluated by the programme. Since curvilinearity of the DNA axis can be visualized as the cumulative effect of small, permanent deflections associated with every base pair, the unbent nature of the pBend2 fragment can be explained on this basis. The effects of the individual base pair wedges cancel each other out, resulting in a straight fragment. This also corroborates with the mobility behavior of the pBend2 fragment as it did not show any

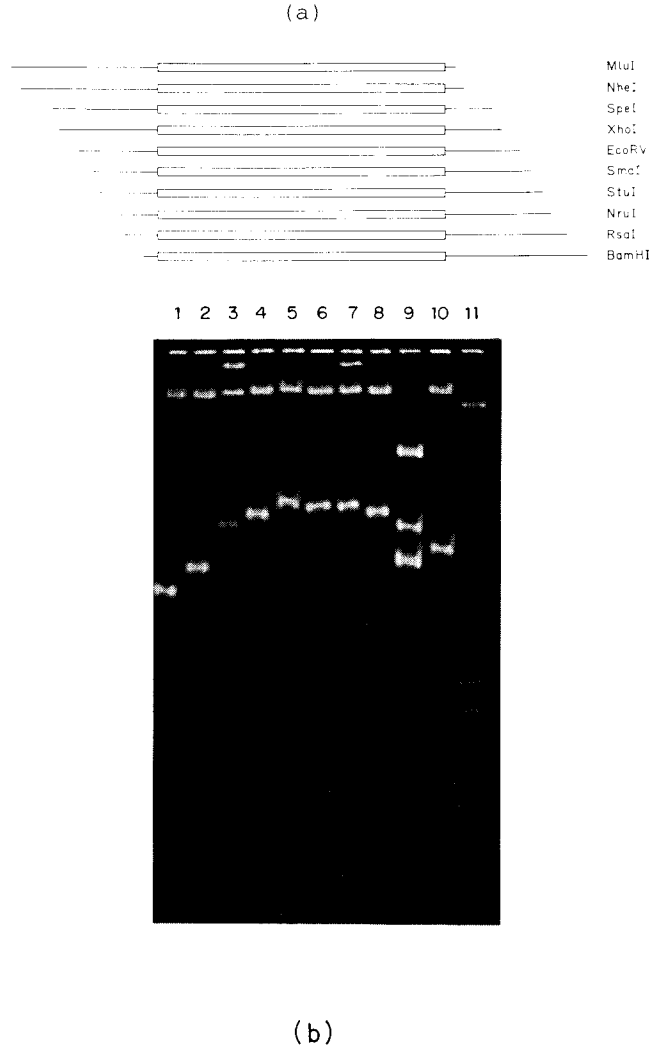

Fig. 3. Analysis of permuted fragments of pMMN32. (a) The permuted fragments generated by different restriction enzymes. (b) Mobility of these fragments in 12% polyacrylamide gel electrophoresis at 4°C. Lanes 1, *Mlu*I; 2, *Nhe*I; 3, *Spe*I; 4, *Xho*I; 5, *Eco*RV; 6, *Sma*I; 7, *Stu*I; 8, *Nru*I; 9, *Rsa*I; 10, *Bam*HI; and 11, pBR322 *Hin*fI digest fragments as markers.

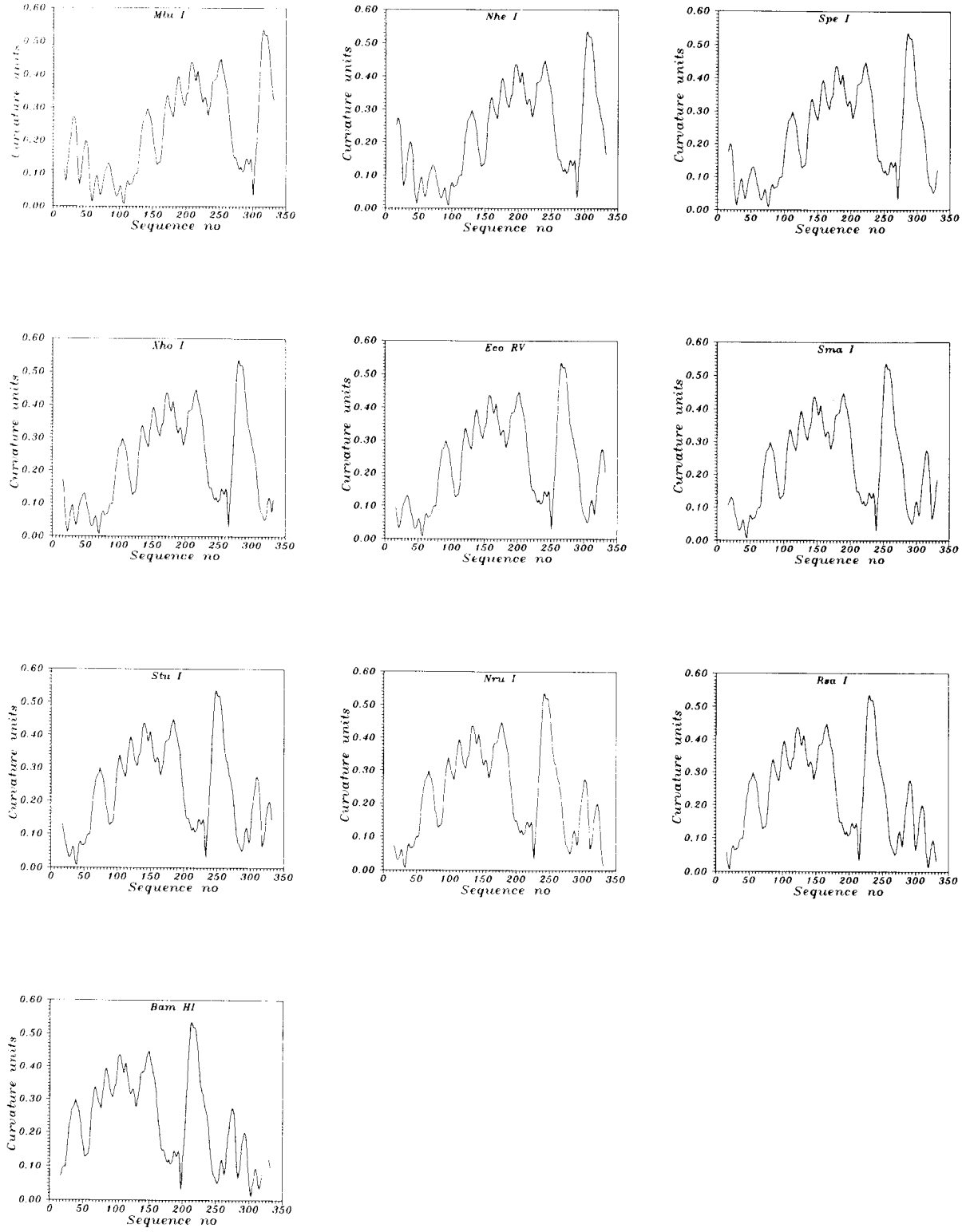

Fig. 4. Curvature map of the permuted fragments of pMMN32. The curvature is given in DNA curvature units and is defined as the average curvature of DNA in the nucleosome core particle, 1/42.8 A (see [18]).

anomalous behavior in gel. This is unlike in a bent DNA fragment where the wedges are in phase with each other, i.e. for two wedge elements (non parallel base pair stacks) to achieve concerted unidirectional deflection of the DNA axis, they must be positioned at specific distances along the double helix.

Permuted fragments were obtained by digesting the recombinant plasmid (pMMN32) with suitable restriction enzymes such that the restriction enzyme does not cut within the K-DNA insert. Fig. 3a shows the position of the insert with respect to the ends of the fragment obtained after digestion. Such

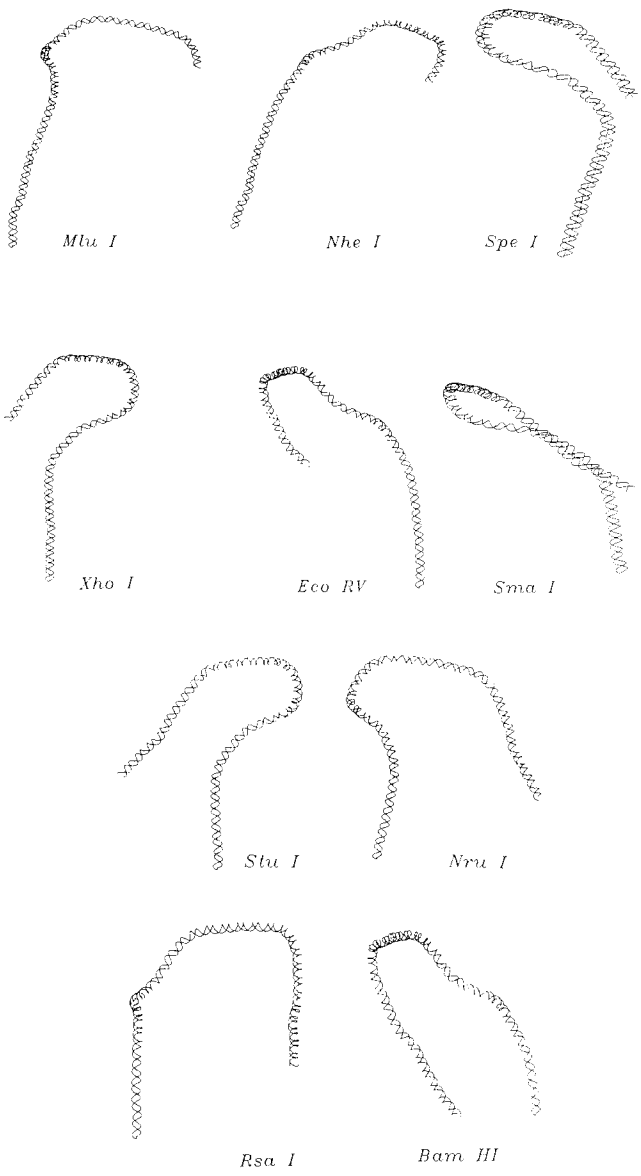

Fig. 5. DNA path of the permuted fragments of pMMN32.

fragments have their wedges at different relative positions, as a result of which these fragments have different degrees of intrinsic curvature. An investigation to this effect was carried out by electrophoresing these fragments in polyacrylamide gel and observing the different degrees with which these reptate. Since their mobility difference is a function of their intrinsic curvature, the fragments with maximum curvature have maximum retardation and minimum mobility. These permuted fragments have conformations that differ because of the variation in the position of the bend relative to the molecular ends. When the bend is near the middle of the molecule, the strongly bent overall shape should encounter more difficulty in snaking through the pores of a polyacrylamide gel than is experienced by a more linear fragment; this is in agreement with the theories of gel electrophoresis [19,20]. Fig. 3b shows the varying degrees with which these permuted fragments reptate. It should be noted that the *Eco*RV is the slowest moving fragment. This is because the insert which itself is intrinsically curved is exactly in the center and the flanking sequence is of the same length on either side. Thus the *Eco*RV fragment is maximally curved and encounters more difficulty in reptating through the pores of the gel during electrophoresis.

Further, with a view to understand the structural features associated with these permuted fragments, they were analyzed theoretically. The curvature map (Fig. 4) of each of these individual fragments clearly shows the curvature shifting from one end of the fragment to the other end. Comparing the curvature maps of the fragments *Mlu*I through *Bam*HI it can be clearly seen that the curvature maximum is shifting from one end to the other. The curvature profile of the *Eco*RV fragment which is maximally retarded shows the curvature maximum to be in the center of the fragment. The DNA path shown in Fig. 5 allows an easy visualization of the different degrees of curvature associated with these fragments. All the fragments shown are projected on the X–Z plane.

Finally we would like to conclude by saying that these observations elegantly demonstrate the effect of the curved insert relative to its position and its effect on the overall curvature of the DNA. The observations provide some explanation to the mobility behavior of these fragments, since gel migration of DNA is a rather complicated phenomenon which is not yet fully understood and lacks satisfactory quantitative description.

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