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Theoretical permutation gel electrophoretic analysis of a curved DNA fragment located in circular permutation

Using the theoretical model for DNA curvature, we analyzed a set of fragments with a curved insert located in circular permutation. The theoretical permutation analysis of each of the cyclically located fragments reveals the presence of a shifting molecular bend locus. The delineation of the molecular bend locus associated with the fragments obtained by a second permutation helps in providing an explanation for the differential mobility behavior of the fragments.

1 Introduction

The conformational properties of the polymorphic molecule DNA are controlled by the nucleotide sequence it contains. Apart from the structural polymorphism based on the variants of DNA secondary structure *viz.* B-DNA, A-DNA, Z-DNA and the parallel stranded and H-DNA, the role of local structural polymorphism has been a subject of intense theoretical and experimental investigations in the recent past [1–4]. The evidence for such local structural polymorphism was found in the crystallographic studies of defined DNA fragments [5]. DNA curvature is an example of such microstructural polymorphism and is now believed to be an intrinsic attribute of certain DNA sequences [1, 6–8]. The existence of such sequence-dependent structural deformation in DNA, which was first identified in the phased $(A)_n$ tracts from the kinetoplast DNA (K-DNA) of *Leishmania torentolae*, is now an established fact [1, 4]. Two classes of models have been proposed to explain the basis of DNA curvature. The wedge model originally proposed by Trifonov [6–9] is based on the assumption that the non-parallelness of the base pairs due to the different dihedral angles between different base pair stacks gives rise to some small sequence-dependent wedges. The axial deflection of the successive wedges combine to form a planer curve and is particularly significant for the AA sequence. The junction bending model [10, 11] identifies the origin of curvature at the conformational transition between locally somewhat different sequence-dependent forms of DNA, such as the deflection of the DNA axis at the junction between B-form and A-form. The phasing of the $(A)_n$ tracts assures that the curvatures of individual bending elements add up to produce a large overall bend in both models. Intrinsic DNA curvature is not limited to sequences containing poly-A runs. Investigations have shown that there are a number of curved DNA fragments whose sequence contain no poly-A tracts [12, 13]. The functional role of intrinsic DNA curvature has been found to be associated with replication, transcriptional regulation and chromatin structure in a number of biological system [15, 16]. There has been evidence that suggests that proteins could use DNA local curvature as structural motifs in the recognition process [17, 18]. The role of DNA curvature in vital biological processes in both eukaryotic and prokaryotic systems has been studied extensively [19–21].

Polyacrylamide gel electrophoresis is by far the most sensitive and convenient method to detect DNA curvature (reviewed in [14]). The linkage between DNA curvature and its anomalous migration in nondenaturing polyacrylamide gel is based on the theoretical investigations of Lerman and Frish [22] as well as Zimm and Levene [23]. They predict that the DNA mobility is directly related to the mean square end-to-end distance. Based on this dependence of mobility on the end-to-end distance, Wu and Crothers [24] proposed the cyclic permutation gel mobility assay to identify the approximate location of putative bending locus. The physical basis of sequence-dependent curvature in DNA was theoretically investigated by De Santis *et al.* [25–29]; they found that the nearest-neighbor differential interaction between base pairs originally proposed by Trifonov [6] could suffice to explain the origin of superstructures in DNA. The model predicts the intrinsic DNA superstructures that were experimentally investigated with a very high degree of reliability. They have also proposed a theoretical method to predict DNA permutation gel electrophoresis from sequence [30]. We had earlier investigated the mobility behavior of a curved DNA fragment when it becomes differentially located on a straight piece of DNA [31]. In the present work we have theoretically analyzed each of these permuted fragments in order to understand their mobility behavior. We have also used the theoretical permutation method [30] to identify the position of bending locus associated with each of the permuted fragments.

2 Theoretical rationale

The model proposed by De Santis *et al.* [25] has been used to understand the superstructures associated with the permuted fragments. The model predicts with good approximation the superstructures of DNA tracts by inte-

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Keywords: Cyclically permuted / Cyclically located / DNA curvature / Theoretical permutation gel electrophoresis

* NCL communication No. 6170

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0173-0835/96/0404-0633 $10.00+.25/0

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AATTCTCCGA AAAAGGGTCA AAAATGGGGA TAAATCCAAA CCATGAGTAG CCTCCGGGTA
GGGGCGTTCT GCAAAATCGG GAAAATTGAT ACAGAAACCC CGTTCAAAAA TCCCCGAAAA
ATCGTATTTT TGGCCTCGGA GCCGTCAAAC TGGGGGTTGG TGTAAAATAG GGCCGGGCGG
CCTGGAATTG GGCCTGAATT TCCTCCCCTG GGCTGGCCGC GCTGC
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Figure 1. The sequence of the kinetoplast DNA (1-222 of minicircle pLdKE3 EMBL access. No. X68026) used to construct the recombinant plasmid pMMN32.

grating the theoretical deviations, as obtained by conformational energy caculations, of the 16 differnt dinucleotide steps from the canonical B-DNA structure [26]. The structural deviations from the canonical B-DNA structure is obtained in terms of the curvature vector $C(n, \upsilon)$ which represents in the complex plane (in modulus and phase) the directional change of the double helix axis between the sequence number n and $n + \upsilon$. The curvature dispersion σ^2 in a given fragment of DNA calculated as the second moment of the angular deviation of the local helical axis from the average direction is linearly correlated with gel electrophoretic retardation. The details of the model are given in the original work of De Santis *et al.* [25]. The modulus of curvature $|c|$ and the curvature dispersion σ^2 for all the permuted fragments have been calculated. The curvature dispersion σ^2 has also been calculated by a cyclic permutation of the sequence. This allows the easy calculation of an analog of the permutation assay for each of the permuted fragments by plotting σ^2 for the cyclically permuted sequences [30].

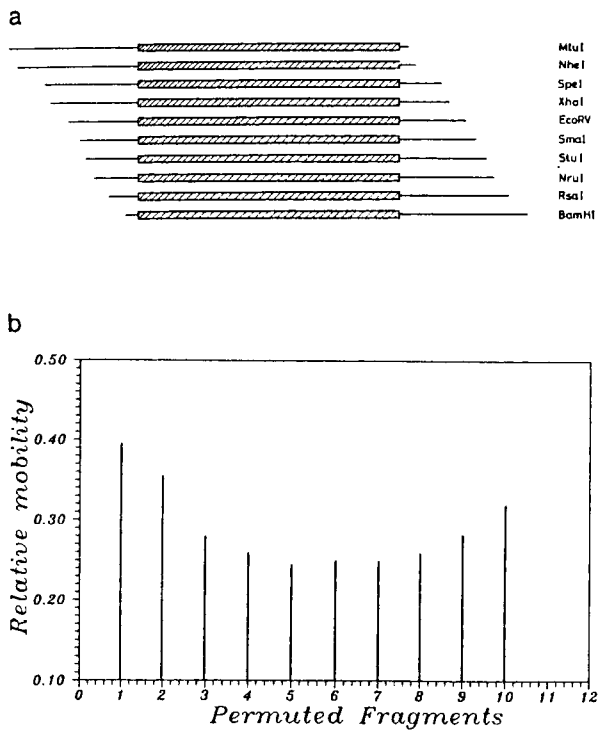

Figure 2. Analysis of permuted fragments of pMMN32. (a) The permuted fragments generated by different restriction enzymes. (b) Relative mobility of the cyclically located fragments in 12% polyacrylamide gel electrophoresis at 4°C. (1) *Miu*I; (2) *Nhe*I; (3) *Spe*I; (4) *Xho*I; (5) *Eco*RV; (6) *Sma*I; (7) *Stu*I; (8) *Nru*I; (9) *Rsa*I; (10) *Bam*HI.

3 Results and discussion

We have used the theoretical model for DNA curvature to analyze a set of permuted fragments wherein a curved insert is located in circular permutation. In order to understand the mobility behavior of a curved DNA when it becomes differently located on a straight piece of DNA fragment, we had in our earlier work [31] studied the mobility behavior of a set of permuted fragments wherein the curved insert was located in circular permutation. These fragments have their flanking sequences permuted while the insert only slides from one end of the fragment to the other. Such a set of fragments was obtained by cloning a K-DNA fragment (Fig. 1, 1-222 of the minicircle pLdKE3 (EMBL access. No. X68026)) which showed anomalous mobility in gel into pBend2, a vector containing 17 restriction sites in a direct repeat spanning the central region containing the cloning site [32]. Experimental details are reported in our previous work [31]. Digesting the recombinant plasmid (pMMN32) so obtained with suitable restriction enzymes, which did not cut within the insert, it was possible to obtain fragments wherein the insert was differentially located with respect to the fragment ends (see Fig. 2a). In this operation, the permutation is effected only on the flanking sequences. A set of such fragments will henceforth be referred to as cyclically located fragments by virtue of the fact that the K-DNA insert is not permuted but is only differentially located. The mobility behavior of these fragments revealed that, although all the fragments that were obtained after restriction digestion were of the same size, they exhibited different degrees of retardation in a polyacrylamide gel [31]. Figure 2b shows the mobility of each of these permuted fragment in a polyacrylamide gel (also see Fig. 1 of [31]).

In order to correlate the differential mobility behavior with the sequence-directed structures in DNA, in the present work we have studied each of these permuted fragments individually. The theoretical model [25] was used to describe the curvature of these permuted fragments. The curvature is represented by a pair of diagrams in which both the modulus and the relative phase calculated from the first nucleotide residue of the curvature vector $C(n, \upsilon)$ are reported versus the sequence number n. In our calculations we have assumed $\upsilon = 31$ (3 turns of the helix). The value of curvature is assigned to the sequence number $(n + 15)$, which is the center of the sequence tract considered. The presence of regions with high curvature is monitored by the corresponding maxima in curvature with a constant phase. The modulus of curvature averaged over three turns of the helix and the phase associated with each of the permuted fragments are shown in Figs. 3 and 4. The results of the calculations reveal the curvature maximum shifting from one end to the other as the curved insert becomes differentially located on the straight DNA fragment. It is also clear from the figure that in the case of the *Eco*RV fragment that is maximally retarded, the curvature maxima is in the center. Moreover, the *Eco*RV fragment has flanking sequences of equal length on either side of the curved fragment (see Fig. 2a). The analysis has also been carried out using the parameter σ^2, the curvature dispersion (averaged over the same window)

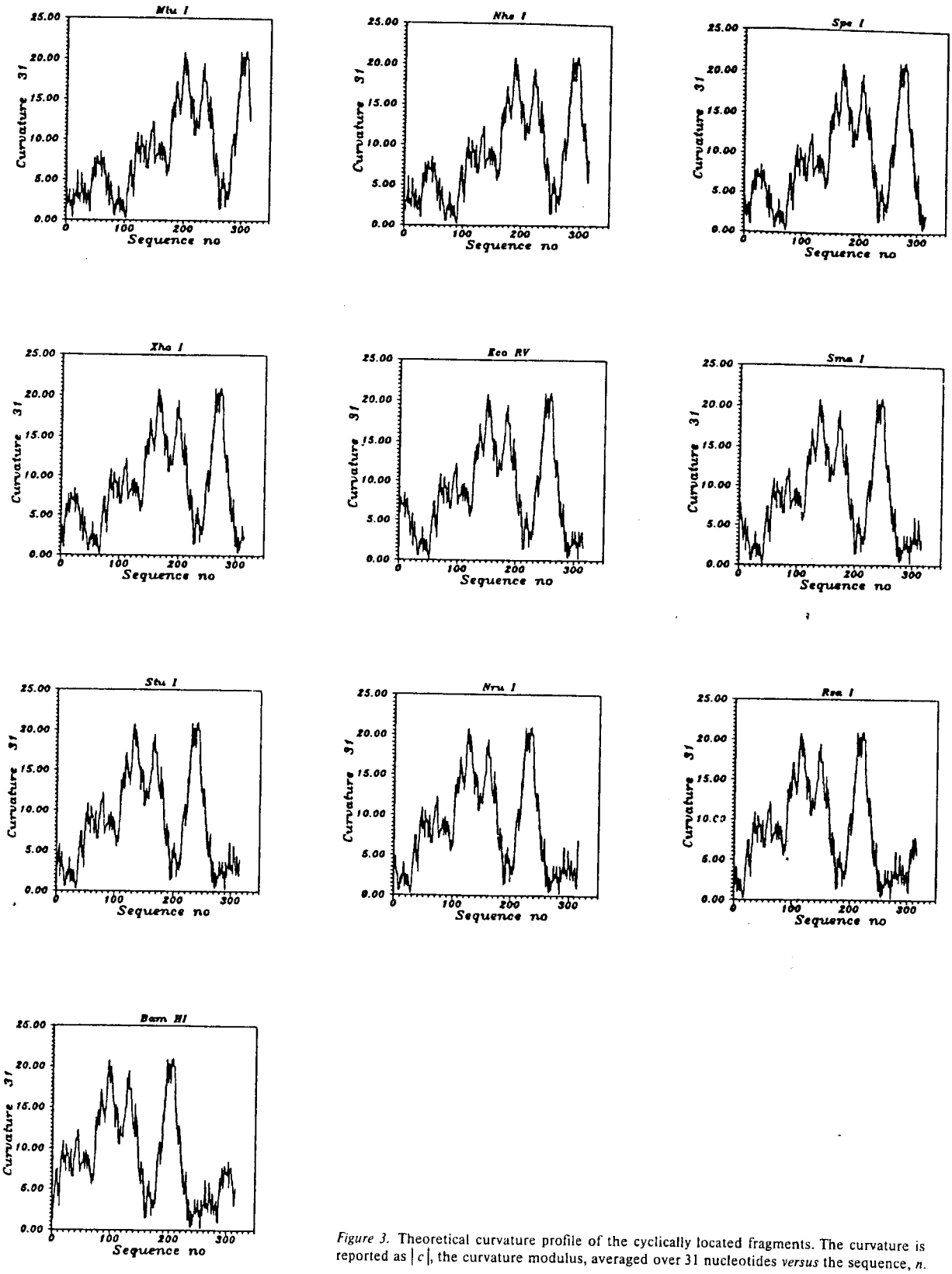

Figure 3. Theoretical curvature profile of the cyclically located fragments. The curvature is reported as $|c|$, the curvature modulus, averaged over 31 nucleotides *versus* the sequence, *n*.

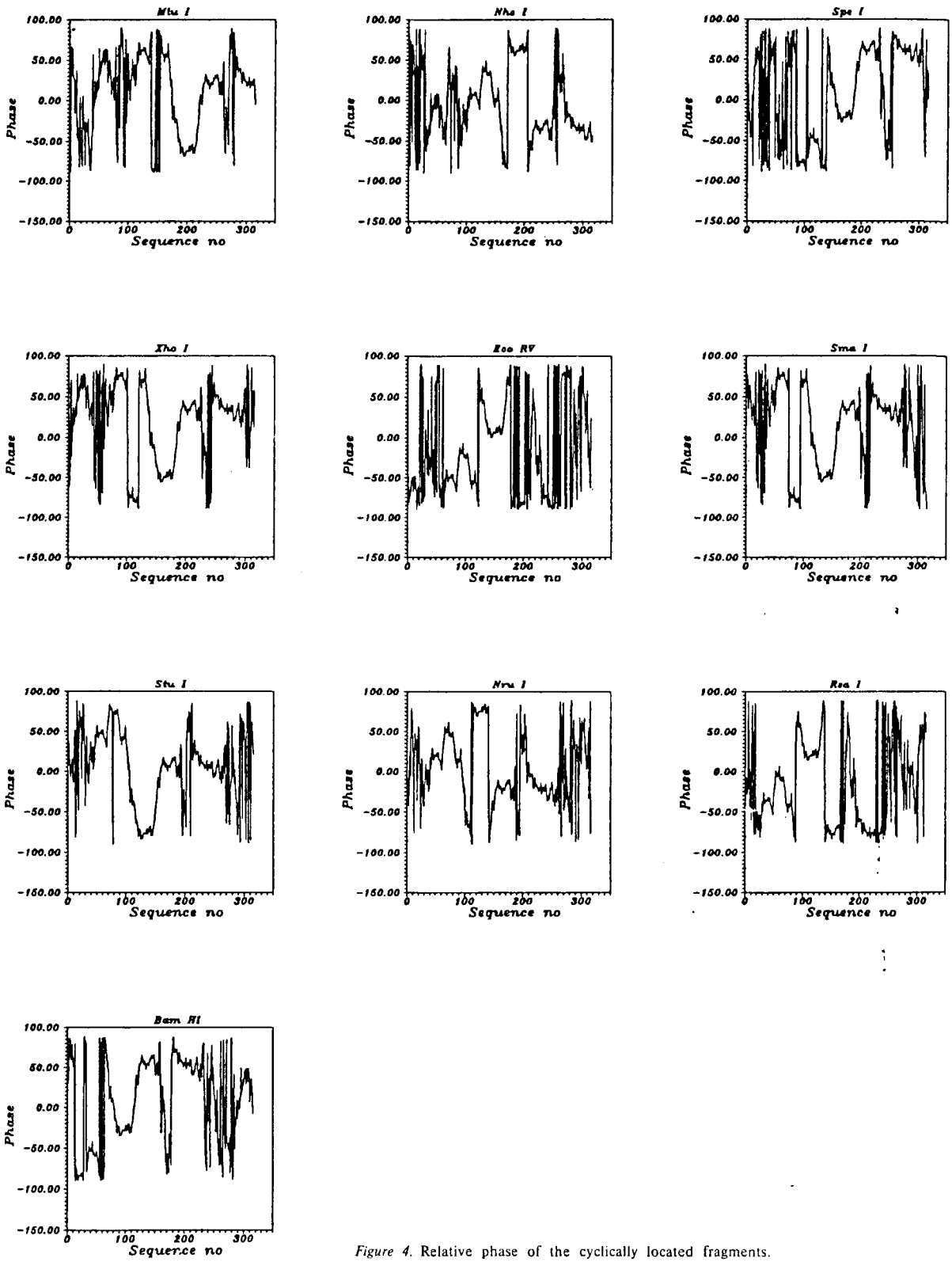

Figure 4. Relative phase of the cyclically located fragments.

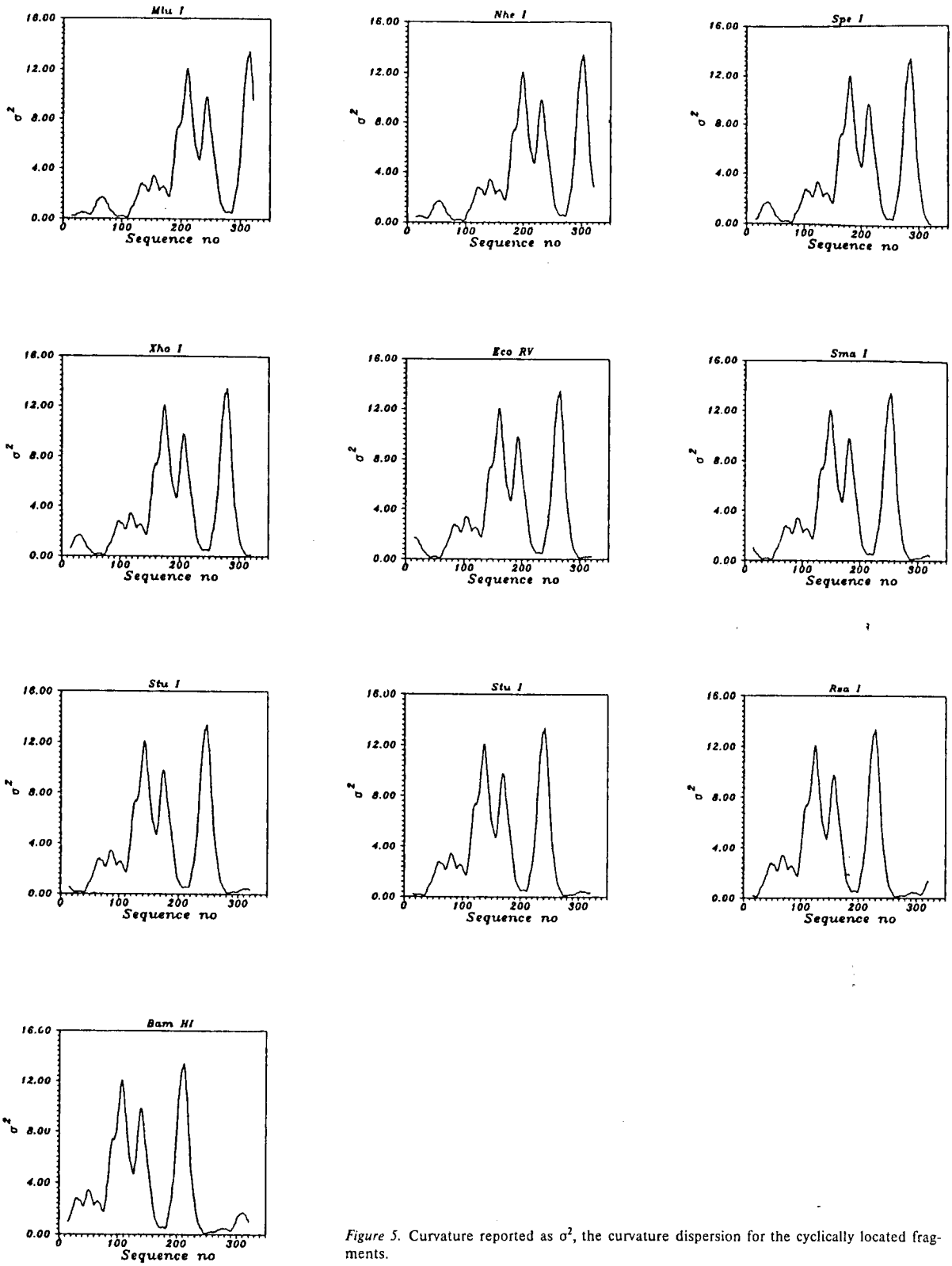

Figure 5. Curvature reported as σ^2, the curvature dispersion for the cyclically located fragments.

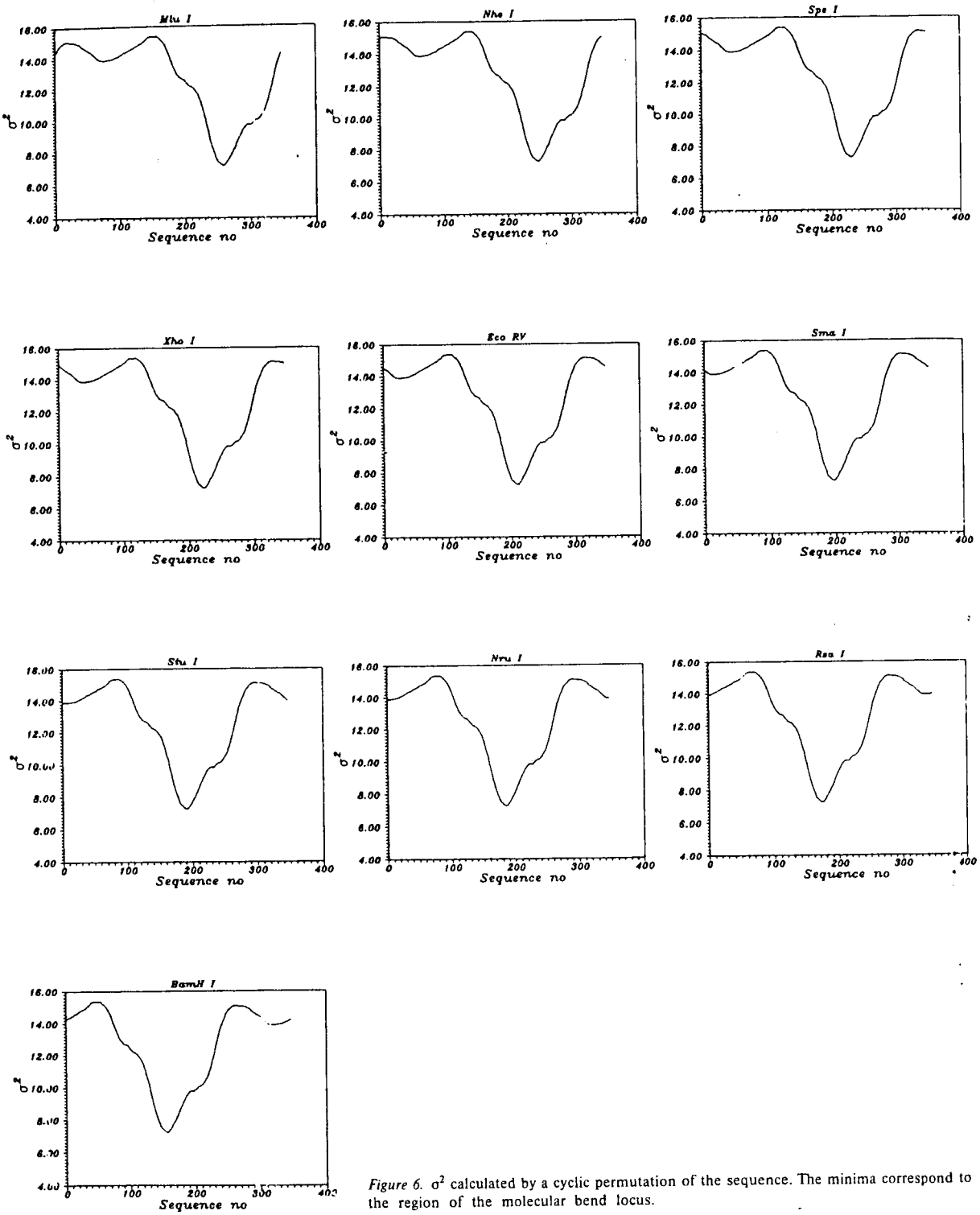

Figure 6. σ^2 calculated by a cyclic permutation of the sequence. The minima correspond to the region of the molecular bend locus.

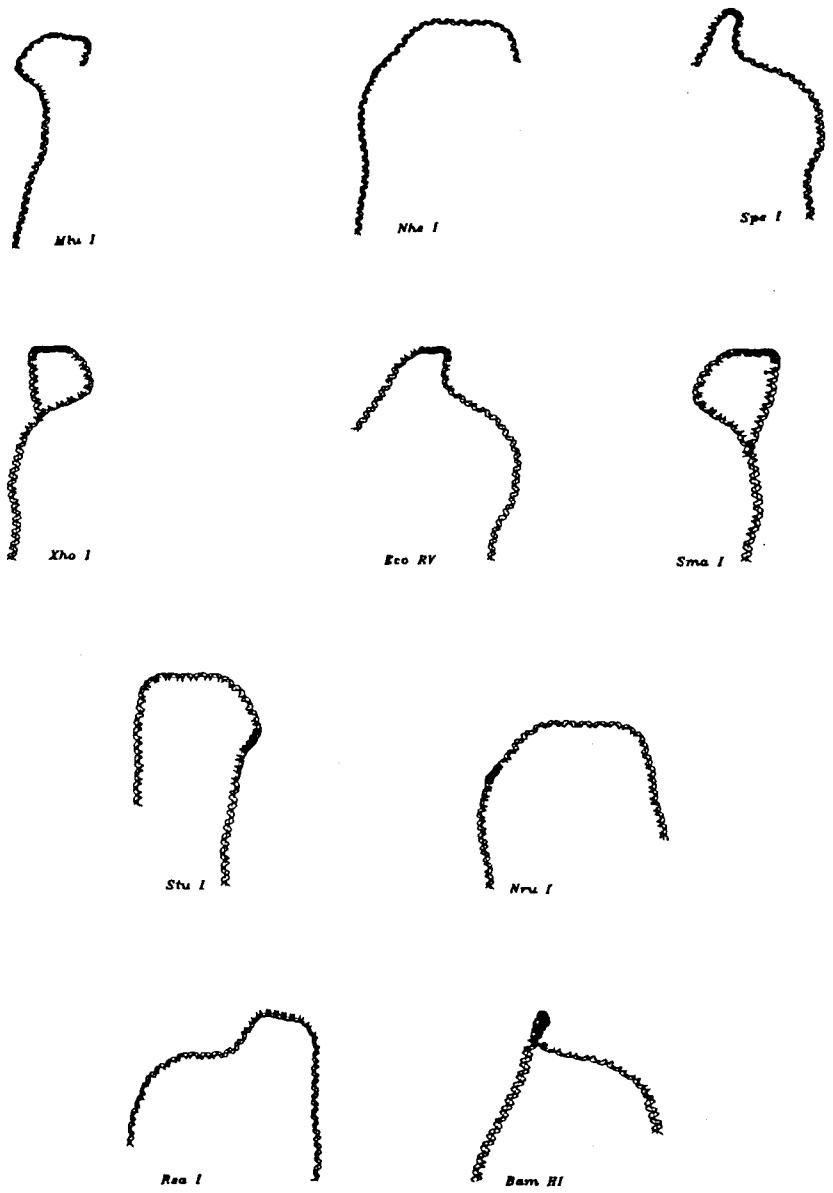

Figure 7. DNA path evaluated by using the energy minimized values of roll, tilt and twist.

according to de Santis *et al.* [25]. Figure 5 shows the plots of the curvature dispersion σ^2 *versus* the sequence number. The curvature dispersion retains the overall characteristic of the curvature profile, but with this method the noise caused by the fluctuation of the curvature along the sequence is cancelled. It allows indviduation of sequences with higher curvature.

It is interesting to compare the mobilities of the MluI and the *Bam*HI fragments. The *Mlu*I fragment has the curved insert located at the right extreme end of the fragment (see Fig. 2a), whereas the *Bam*HI fragment has the curved insert located at the left extreme end. In view of the same flanking sequences and the symmetry (see Fig. 2a) one would expect them to move with the same mobiltiy. The *Mlu*I fragment, however, moves faster than the *Bam*HI fragment. This indicates that the *Mlu*I fragment should have its main bend site located more towards the end of the fragment than the *Bam*HI fragment. An investigation to this effect was carried out by calculating σ^2 for each of these fragments, but this time by cyclically permuting the entire sequence. Since σ^2 has been found to be linearly correlated with gel electrophoretic retardation, quantified as the ratio between apparent and real lengths, calculating σ^2 for cyclically permuted sequences would be a theoretical analogue of the cyclic permutation gel electrophoresis assay. Moreover, only the trend in the retardation against the restriction

site along the sequence is required for localizing the molecular bend locus. The set of cyclically identical fragments has the putative bending sequence elements positioned from near the center to near an end by the cyclic permutation of all bases in the sequence. These fragments are related by operations of circularization, ligation and recutting the circle at a new location. The mobility goes through a minimum as the bending locus passes through the center of the sequence [14, 24]. Calculating σ^2 by cyclically permuting the sequence permits us to locate the molecular bend locus for each of the cyclically located fragments. Doing the permutation assay experimentally becomes difficult when the fragment under study does not contain enough unique restriction sites within it. However, the theoretical analogue of the permutation assay [30] can be used as an alternative to locate the main bend site. At this point it is important to emphasize the importance of doing a second permutation of the already permuted fragments (with respect to flanking sequences). The experimental permutation (cloning and digesting the insert in pbend2 vector), permutes only the flanking sequences and not the insert *per se*. The second permutation (theoretical) cyclically permutes the entire sequence (the flanking as well as the insert). This permits us to locate the molecular bend locus of each of the permuted fragments.

The results of the theoretical permutation assay are given in Fig. 6. It is evident by comparing the plots *Mlu*I through *Bam*HI that the molcular bend locus also shifts from one end of the fragment to the other as the fragment beocmes differentially located on the straight piece of DNA. It is worth comparing the trend in the mobility of these fragments with the relative position of their molecular bend locus. The permutation diagram of the *Mlu*I fragment reveals the main bend site associated with it to be lying at the extreme end of the fragment, whereas the permutation diagram of the *Bam*HI fragment show the location of the main bend site to be lying closer to the center of the fragment as compared to the *Mlu*I fragment. These results are also in agreement with the mobility behavior of these fragments. The fragments with their main bend site lying closer to the center of the fragment would be more retarded than the fragments whose main bend site is lying more towards the end of the fragment. The other fragments also have their bend locus at different positions by virtue of the curved insert being differentially located on the fragment and thus exhibiting different degrees of mobility. The superstructures associated with each of these fragments can be easily visualized by comparing the DNA path (Fig. 7), evaluated by the CURVATURE program [33] using the energy minimized values of roll, tilt and twist as obtained by De Santis *et al.* [26].

4 Concluding remarks

The theoretical model based on the nearest-neighbor interaction is capable of translating deterministic fluctuation of base sequence in pieces of information on DNA superstructure. The σ^2 obtained by the cyclic permutation of the sequence, which allows an easy calculation of an analogue of the permutation assay, helps in localizing the bend centers associated with the cyclically located fragments, which are permuted fragments with respect to flanking sequences. The bend centers associated with the cyclically located fragments, obtained by a second permutation of the entire fragment, provide a basis for explaining the mobility behavior of the cyclically located fragments. We hope this study will contribute to the understanding of the anomalous behavior of curved DNA fragments.

The authors thank the referees for their comments on the previous version of the manuscript. The authors also thanks Prof. E. N. Trifonov and E. Shepigelman for the CURVATURE program. The help rendered by Prof. P. De Santis in understanding his model is greatly acknowledged. TMN thanks Prof. Trifonov for having introduced a novice to the complexities of DNA sructure. This work is supported by the Council of Scientific and Industrial Research (CSIR), India.

Received February 18, 1995; in revised form December 18, 1995

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