EFFECTS OF DNA PRIMARY STRUCTURE ON TERTIARY STRUCTURE

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1. Introduction

Much recent information has been obtained about specific areas of DNA molecules which may serve as recognition regions for proteins because of micro-variation in primary, secondary or tertiary DNA structure. In the case of primary structure evidence has been presented to show that homopolymeric regions are present in some animal DNA molecules [1], while in the field of secondary structure Bram [2, 3] has shown that a wide range of variations on the classical Watson–Crick structure can exist. Tertiary structure variations have been detected as palindromes or inverted repetitions in mammalian and in viral DNA molecules. It seems relevant therefore to ask whether these variations can affect the three dimensional structure of normal DNA molecules in solution when they are exposed to slight superhelical torsion. It is generally accepted that non-superhelical DNA molecules in solution have a worm-like coil structure with a persistence length of 41 nm and that this is not affected by variations in primary structure [4,5]. Superhelical DNA molecules however have a known tendency to unwind and could be expected to unwind preferentially in certain areas with a concomitant effect on their tertiary structures exposing putative 'active site' regions analogous to the anticodon loop of tRNA or the active centre cleft in enzymes. Such areas would obviously have an altered affinity for any proteins which show differential affinities for single and double stranded DNA.

*Abbreviation: ϕX174 RFI DNA, circular duplex superhelical replicative form of ϕX174 DNA. The DNA molecules used were ϕX174 RF and SV40 which is almost the same size as ϕX174 RF but from a mammalian virus.

We have already made a detailed investigation of the structure of ϕX174 RFI DNA using a conventional light source [6, 7] and have shown that within the normal range of superhelix density it exists as a Y-shaped structure with arms of approximately equal lengths and 34 nm diameter. This requires that the stiff double helix make a very tight turn in three places at the ends of the arms of the Y and suggests that the molecules with regions which preferentially unwind because they are A T rich or have some palindromic character may prefer to have such regions located at the ends of the arms of the Y. Nonetheless the symmetry of the structure also suggests that this may arise from some general topological property of DNA rather than the primary sequence. If the number and length of the branches is dictated by primary structure rather than any more general parameter it seems likely that different DNA molecules from other sources will adopt differing conformations in solution with the numbers and lengths of the arms being determined by the disposition of the early denaturing and palindromic regions on the genome. The work reported in this paper was undertaken to test such a hypothesis.

2. Methods

ϕX174 RFI DNA was made as described previously [6]. SV40 DNA was extracted from BSC 1 cells, infected with SV 40 virus at a low multiplicity (0.01 pfu/cell), by the method of Hirt [8] and purified by CsCl-propidium iodide centrifugation. Such preparations contained less than 1% defective SV 40 DNA molecules.

Light scattering experiments were performed on

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an instrument constructed by Precision Devices Ltd., Malvern, which used a helium neon laser as light source. This gives increased sensitivity on conventional instruments and can be used with very small samples at angles down to 25°C. Calibration was with Ludox colloidal silica as described previously [6]. Clarification was with 0.45 μm millipore filters. Dust contamination was reduced on previous experiments by the use of dust free cells obtained by drying the inverted cells over dust free water in a hot oven. The DNA was analysed by sedimentation before and after the light scattering experiments to test for breakdown. DNA concentration and superhelix densities were determined as described previously [6].

The data were processed in an IBM 370/158 computer which generates the toal interference curves compatible with all models having the experimentally measured root-mean square radius and molecular weight. The interwound, toroidal and Y shaped models were treated mathematically as described previously [6, 7].

3. Results

Typical Zimm plots of the two DNA molecules are shown in fig. 1. The two DNA molecules are very close in size and suitable for comparison with respect to shape. The root mean square radii are also similar so that the shape difference is little reflected in this parameter which is derived from the initial slope of the Zimm plot. However at higher angles the Zimm plot for SV 40 levels off and becomes much flatter than that of ϕXRF DNA. This is shown more clearly in fig. 2 where the $P(\phi)^{-1}$ curves for the two DNA molecules are drawn and compared with theoretical models constructed from the experimental molecular weights and root mean square radii. The toroidal models are models in which no specific segment of the DNA experiences any particular strain and the double helix is under uniform torsion throughout, the straight inerwound model shows two areas where the double helix experiences extreme torsional stress and the Y shaped models have three areas of torsional stress, one at the end of each arm. It can be seen that whereas ϕX RF DNA fits the curve for a symmetrical Y shape as has been shown previously [7], SV40 DNA fits a pattern for an asymmetric Y shape. It has been shown [9] that SV 40 has two sites susceptible to S1 nuclease and it is possible that these correspond to the ends of two of the arms. However the existence of a third arm is not greatly surprising since it is possible that the DNA can double back on itself at such places and form regions with minimal single stranded areas. It has been suggested that the number of superhelical turns determined by ethidium titration may be too high because the DNA is partially unwound in solution [10]. Conversely it has also been suggested that the number may be too low [13]. Clearly the number represented in fig.2, may therefore be wrong but this does not affect the basic conclusion that two DNA molecules have arms of different length as summarised in table 1. An alteration in the number of superhelical turns present would affect the diameter of the arms but not the length and the conclusion that one molecule is symmetric and the other asymmetric remains.

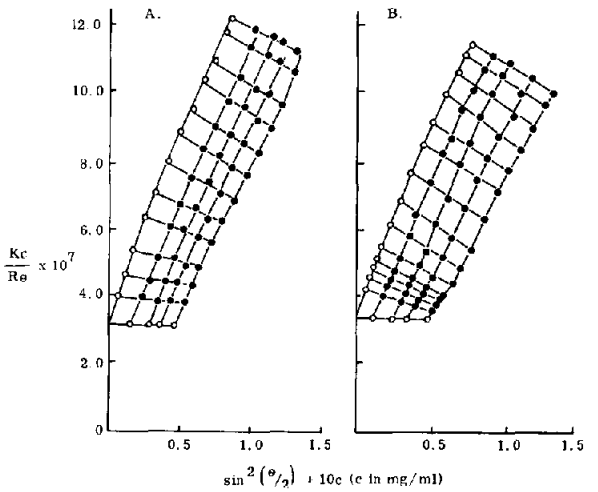

Fig.1. Typical Zimm plots of SV40 and ϕX 174 DNA in BPES buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA and 0.179 M NaCl, pH 6.8) (A) ϕX174 DNA; (B) SV40 DNA.

Much of the current information about DNA structure has been obtained by electron microscopy, using the protein monolayer technique of Kleinschmidt and Zahn [12]. The spreading forces involved can obviously distort the molecular conformation with respect to the solution structure and the reduction in charge and persistence length caused by the binding of the basic protein in those techniques can affect the capacity of the DNA to bend at the end of the branches. The ex-

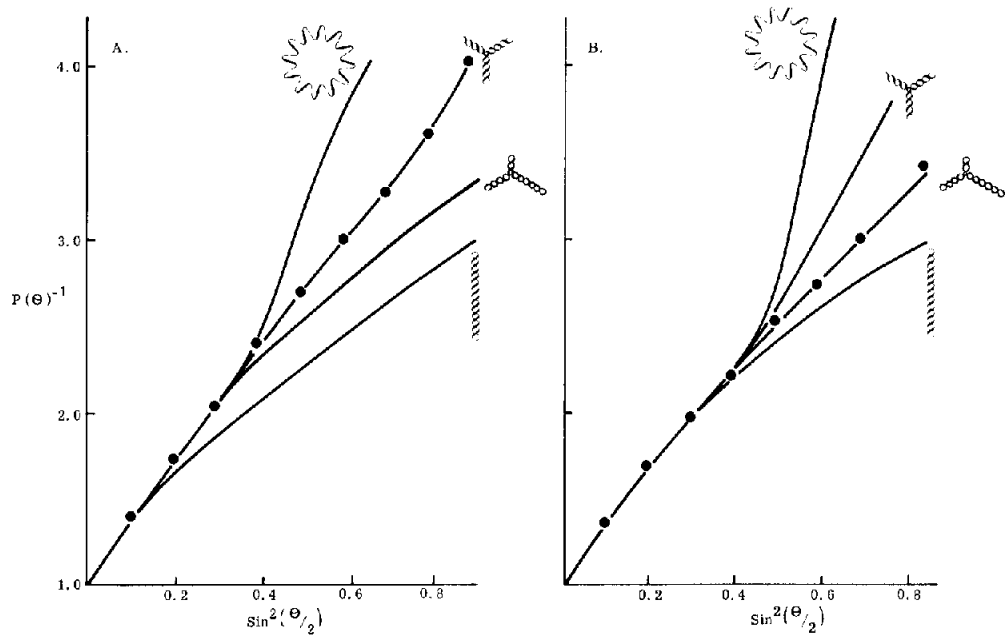

Fig.2. $P(\theta)^{-1}$ curves of ϕX174 RF and SV 40 DNA together with visual representation of structural models. The top model in each case is the toroidal model and the bottom one the straight interwound model. (A) ϕX174 RF; (B) SV40.

periments described here allow the accuracy of the data from electron microscopy to be tested in solution and to some extent complement that work. In addition structures which are three dimensional in nature such as tetrahedra and toroids can be investigated. Such structures are difficult to see in electron micrographs.

We interpret this data as providing evidence for two related hypotheses. Firstly that three dimensional structure of superhelical DNA is specific and dependent on the primary structure. Secondly it is possible to envisage in solution unwinding sites on DNA which may be termed 'active sites' and which may affect the expression of the viral genome in the same way as the three dimensional structure of viral RNA affects its transcriptional properties [11].

Two possible suggestions can be made about the primary structure of such sites. They may be rich in AT regions and consequently they may have a greater tendency to unwind for energetic considerations. Alternatively they may have some ability to double back on themselves in a palindromic fashion and hydrogen bond with other bases on the same strand. A combination of the two possibilities may be the most realistic suggestion. A separation of strands in an AT rich region where some compensation for the loss in inter-

Table 1
Size and shape parameters of ϕX 174 RF and SV 40 DNA

	Mol. Wt	Root mean square radius	Arm diameter	Arm lengths 1	2	3
ϕX174 RF	3.2×10^6	95 nm	34 nm	172 nm	172 nm	172 nm
SV 40	3.0×10^6	93 nm	32 nm	154 nm	231 nm	77 nm

strand base pairing may be achieved by intrastrand base pairing. To some extent therefore, the two alternatives cannot be regarded separately. However it is possible to investigate the possibility of palindromic regions and AT rich regions separately by well tried techniques which may further extend our understanding of DNA tertiary structure.

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