Biochem. J. (1976) **155**, 101–105
Printed in Great Britain

Conformational Analysis of Deoxyribonucleic Acid from PM2 Bacteriophage

THE EFFECT OF SIZE ON SUPERCOIL SHAPE

By AILSA M. CAMPBELL
Institute of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

(*Received* 29 *September* 1975)

Laser light-scattering studies of bacteriophage PM2 DNA showed the molecule to have mol.wt. 5.9×10^6 and root-mean-square radius 125 nm at an ionic strength of 0.2 mol/litre. Computer-generated curves compatible with these data were compared with the experimental interference curve for several structural models of the molecules. The data fit best to an asymmetric four-armed planar molecule in which all four arms emerge from or close to the one area of the molecule. This contrasts with the smaller DNA molecules investigated, which have shown a three-armed molecule, whose symmetry varies with primary structure.

The existence of denatured regions in superhelical DNA has been well established in recent years. Superhelical DNA molecules experience a strong topological restraint, which is only relieved by unwinding of the secondary structure, and certain regions of the DNA unwind preferentially. The use of chemical probes together with single-strand-specific nucleases and unwinding proteins has shown that there are at least three and possibly four early unwinding sites on polyoma DNA (Germond *et al.*, 1974; Monjardino & James, 1975), that there are up to four sites on bacteriophage PM2 DNA (Jacob *et al.*, 1974) and that there are at least two on virus SV 40 DNA (Morrow & Berg, 1973; Beard *et al.*, 1973). The number of sites on virus SV 40 DNA has also been shown to be sensitive to small changes in the ionic strength of the medium, presumably because of the exceptional susceptibility of the compact superhelix to ionic-repulsion forces. The distribution of such sites around the circular genomes of these DNA molecules is as specific to the DNA source as is the number of sites, and consequently it seems likely that each DNA molecule has a unique secondary structure when under superhelical restraint.

One of the topological problems of tertiary-structure folding in supercoils has been the apparent necessity for the stiff double helix to bend through very tight angles to form the branched and linear structures, which many such molecules appear to adopt. The existence of single-stranded flexible regions at such turning points was first suggested by Crick (1971). In the case of a molecule with a circular genome and several such regions it can be expected that the molecule may take up a conformation that will place such regions at the sharp bends in the molecule. A logical consequence of the specificity of number and position of single-stranded areas is then that the three-dimensional structure of each DNA may also be unique under superhelical torsion.

Light-scattering is a technique that allows the solution conformation of molecules in this size range to be investigated. In previous work we have established the mathematical background for such a conformation analysis and have established a three-armed structure for ϕX174 RF I DNA* (Jolly & Campbell, 1972*b*). The effect of variation of superhelix density induced by changes in dye binding and temperature has also been reported (Campbell & Jolly, 1973). Investigation of two DNA molecules from different sources, but of the same size, has shown that each has a unique structure (Campbell & Eason, 1975). In the present paper the effect of size has been investigated. The only superhelical DNA molecule known to exist in the suitable size range is that of PM2 virus. This DNA is double the size of that of SV 40 and polyoma viruses and bacteriophage ϕX174. The work was undertaken to find out whether the doubling of size led to a simpler structure, as the potential for more even spreading of torsional restraints could result in a balanced toroidal structure, or whether the increase in size led to a greater complexity of branching pattern. The symmetry of the structure was also of considerable interest because of the asymmetry in the early denaturation map (Jacob *et al.*, 1974).

* Abbreviation: ϕX174 RF I DNA, intact circular duplex intracellular replicative form of bacteriophage ϕX174 DNA.

Experimental

Virus PM 2 DNA was isolated by the method of Espejo & Canelo (1968) and the two forms were separated by CsCl–propidium iodide equilibrium centrifugation (Hudson *et al.*, 1969). The number of superhelical turns as determined by ethidium titration (Waring, 1970) was 30±2 in BPES buffer (6mM-Na_2HPO_4, 2mM-NaH_2PO_4, 1mM-disodium EDTA and 0.179M-NaCl, pH6.8). However, this value can be regarded as unreliable for two reasons. First, there is considerable evidence to suggest that superhelical DNA molecules are unwound in solution to some extent and hence the number of superhelical turns determined by titration do not reflect the actual number present in solution, which will be smaller. However, there is also uncertainty as to the basis of the ethidium titration, and the number of superhelical turns has been suggested to be very much greater than was previously thought (Wang, 1974; Pulleyblank & Morgan, 1975). It seems highly doubtful if an accurate estimate of the true number of superhelical turns in this medium can be made at this time. This has virtually no effect of the conclusions obtained by light-scattering when arm lengths are being considered, but does affect the packing of the superhelical turns into these arm lengths and consequently the diameter of the superhelix.

Light-scattering experiments were performed on an instrument manufactured by Precision Devices Ltd., Malvern, Worcs., U.K., by using a 30mW helium–neon laser as light-source at 632.8nm. Measurement of the scattered light was by photon counting with light-comparison stabilization by a monitor photomultiplier to correct for laser output drift. Calibration was with Ludox colloidal silica as described before (Jolly & Campbell, 1972*a*). Dust was removed by the use of 0.45μm Millipore filters and also by the use of dust-free cells obtained by drying inverted cells over dust-free water in a hot oven.

The computer models were generated on an IBM 370/158 computer by using the experimentally obtained values for molecular weight and root-mean-square radius. The various models tested have all been described before (Jolly & Campbell, 1972*a*; Campbell & Jolly, 1973), except for those with four arms. Interpoint distances in such models were obtained by expansion of the technique used with the Y-shaped model by using the equation:

$$r_{ij} = (r_{i0}^2 + r_{j0}^2 - 2r_{j0}r_{i0}\cos m)^{\frac{1}{2}}$$

where m is the angle between branches, and r_{i0} and r_{j0} are the distances of the ith and jth segments from the point of intersection. For interpoint distances on the same arm the rod model was used. This gave the absolute value of the vector between points i and j for application in the light-scattering equation:

$$P(\theta) = \frac{1}{N^2}\sum_{i}^{N}\sum_{j}^{N}[\sin(hr_{ij}/hr_{ij})]$$

where h is $4\pi\sin(\theta/2)/\lambda'$ and r_{ij} is the absolute value of the vector. N is the number of scattering points in the molecule and θ the scattering angle. λ' is the wavelength of light in the medium. The DNA was assumed to be in the B structure with a linear mass density of 1950 daltons/nm (Campbell & Lochhead, 1971).

DNA concentration was measured in a Guilford spectrophotometer. Analysis of the results by the method of Hirschman & Felsenfeld (1966) gave a molar extinction coefficient with respect to phosphorus of 6620 (S.D.±2%).

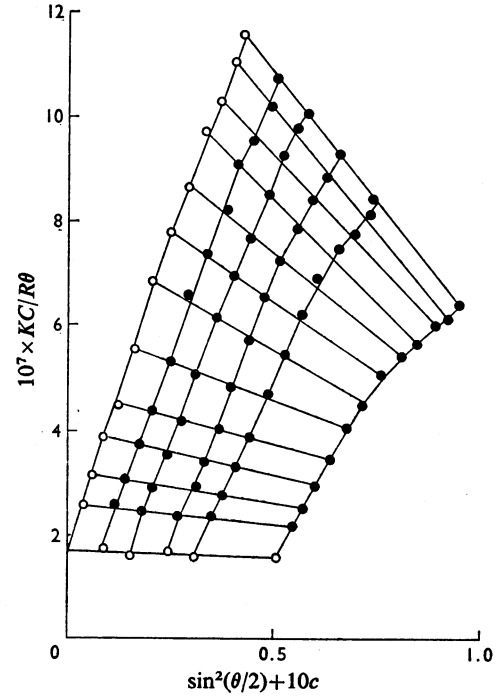

Fig. 1. *Typical Zimm plot of virus PM2 superhelical DNA*

The vertical extrapolation is to zero angle and the horizontal extrapolation to zero concentration, which gives the root-mean-square radius (radius of gyration) from its initial slope and the total interference curve under ideal conditions when all the angles are taken into account. The angular range is from 30° to 135° and the concentration range from 5 to 50μg/ml. The information obtained from the plot is as in Campbell & Jolly (1973), except that the use of vertically polarized laser light leads to a doubling of the optical constant K to $4\pi^2 n^2 (dn/dc)^2/N\lambda^4$ where n is the refractive index of the solvent, dn/dc the refractive increment of the DNA, N is Avogadro's number and λ is the wavelength of the light; c is expressed in mg/ml.

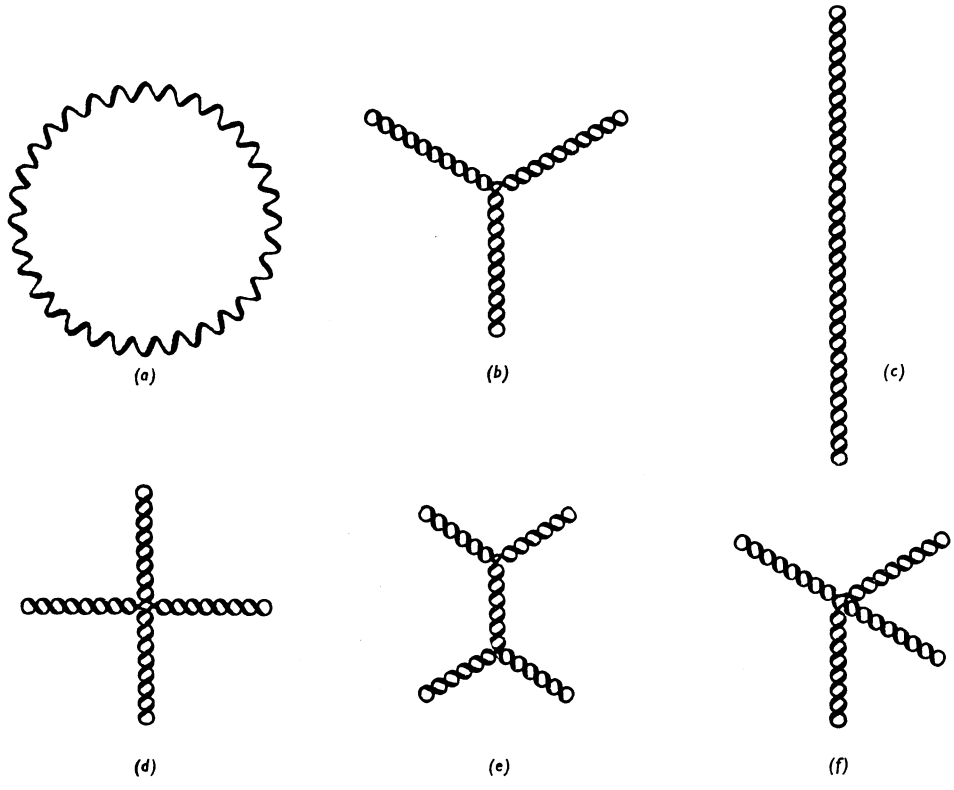

Fig. 2. *Models for virus PM2 superhelical DNA structure*

All the models shown have 30 ± 2 superhelical turns. (*a*) Toroidal structure. All segments of the DNA are under the same torsional and bending strain. (*b*) Y-shaped model. (*c*) Straight interwound model. (*d*) Cross-shaped model. (*e*) Double-Y model. (*f*) Tetrahedral model. All models are shown in their symmetrical forms.

Results

The mol.wt. of virus PM2 DNA was found from the Zimm plot (Fig. 1) to be $5.9\times10^6\pm0.1\times10^6$ and the root-mean-square radius 125 ± 10 nm. These two parameters define the contour length of the DNA (3025 nm) and the basic structure of each type of model and were used in all the computer-generated curves. Fig. 2 shows the various types of model tested.

Toroidal model

One of the advantages of light-scattering is that a three-dimensional structure that would be difficult to envisage by electron microscopy can be investigated. The toroidal model is just such a structure. Unlike any of the branched structures its interference curve is very sensitive to variations in superhelix density, and in view of the uncertainty of the value of this parameter a variety of superhelix densities were tested. However, Fig. 3 shows that the model cannot fit the data unless the number of superhelical turns is well below 15 and can therefore be discounted.

Models with three branches or less

Both the straight-interwound and Y-shaped models can be ruled out by inspection of the data in Fig. 4. The straight-interwound model is not represented but would correspond to a Y shape with one very long arm and two very short arms and would clearly give far too flat an interference curve. The Y shape itself even at its most symmetrical also gives too flat an interference curve.

Tetrahedral model

One of the simplest extensions of the three-branched Y-shaped model is a model with four arms in tetrahedral formation with the angle of 120° between each arm maintained. This implies that the arms must emanate from a common vertex at the centre of the tetrahedron, and the shortening of one arm would necessarily mean the lengthening of another. In view of the evidence, which suggests that the ends of the arms of the branched structures may be specific regions in the DNA sequence, too many restraints may be imposed on the topology by this struc-

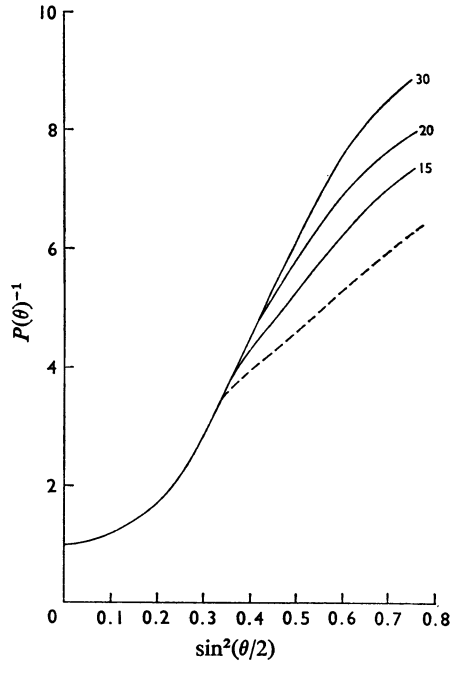

Fig. 3. *Calculated scattering curves for toroidal forms of virus PM2 DNA*

Theoretical curves show 30, 20 and 15 superhelical turns respectively. The root-mean-square radius is 125nm in each case. ----, Experimental line.

ture. However, it is one that again may not be easily investigated by electron microscopy because of its three-dimensional nature. The data in Fig. 4 show it to be an unlikely conformation for virus PM2 DNA.

Double-Y and cross-shaped models

There is no empirical reason to expect the DNA molecule to take up a cross-shaped structure, since one would expect mutual repulsion between the arms to force the angles between the arms to the higher value of 120° in a tetrahedron. However, the cross-shaped model represents the simplest test of a double-Y shape with a very short central arm. The experimental data fit well to this model or indeed to the double Y when the central arm is allocated a very small proportion of the contour length. In either case the model shows asymmetry with two short arms on the one side of the model and two long arms on the other side.

If the planar double-Y model with a short central segment is taken as the best-fitting model the diameter of the arms becomes 26nm at 32 superficial turns. A doubling of the number of superficial turns (Wang, 1974; Pulleyblank & Morgan, 1975) would imply a diameter of 14nm. In either case the diameter is well below the statistical segment length of 82nm and a very tight bending at the end of the arm is implied.

Discussion

The problem of tertiary DNA folding is complex and fascinating. The DNA in viruses and in chromatin is exceptionally compact compared with the solution conformation, which shows a B secondary structure and a statistical segment length of 82nm. Suggestions for the folding of DNA have encompassed a range of superhelix types, 'kinks' in the DNA (Crick & Klug, 1975) and collapsed structures such as ψ DNA (Maniatis *et al.*, 1974). Light-scattering provides a method for studying this folding in solution, though in a limited size range. The structure indicated for virus PM2 DNA by this study shows that increased branching with size can occur. The fact that all the branches emanate from the same area of the molecule may be a coincidence in the case of virus PM2 DNA, or may be a general structural feature. Obviously for Y-shaped molecules the structure of the vertex is not in question. The asymmetry of the structure reflects the early denaturation map of Jacob *et al.* (1974), though no direct parallel can be drawn. It is possible that other factors such as sequences of inverted repetition may modify the folding sites on the three-dimensional structure so that the centre of the denaturing area is not necessarily at the exact tip of the superhelix branch.

One of the major defects of conventional electron microscopy of such DNA molecules has always been that the combination of cytochrome *c* with the DNA may have created a totally different shape of molecule. The spreading forces could further distort the DNA from the solution conformation. The experiments reported in this and previous papers indicate that in fact electron microscopy has given a good indication of superhelix structure, but that the combination and spreading procedures have masked the specificity of tertiary structure.

The true existence of the great variety of structures visible in electron microscopy has always been improbable, as such conformations are unlikely to be identical in minimum energy states. The Boltzmann distribution predicts that if two likely conformations have only 8.4kJ/mol difference in energy the more stable conformation would be favoured by 98% of the molecules in the solution population.

The existence of heavily bent or kinked areas of DNA at the ends of the arms may, of course, be a complete artifact owing to the separation of the virus from its protein coat. However, it is possible that such areas may potentiate some viral function. The preferential reaction of supercoils with unwinding proteins, RNA polymerase (Botchan *et al.*, 1973) and single-strand nucleases, all at specific sites, suggests that if viral DNA should retain its superhelical con-

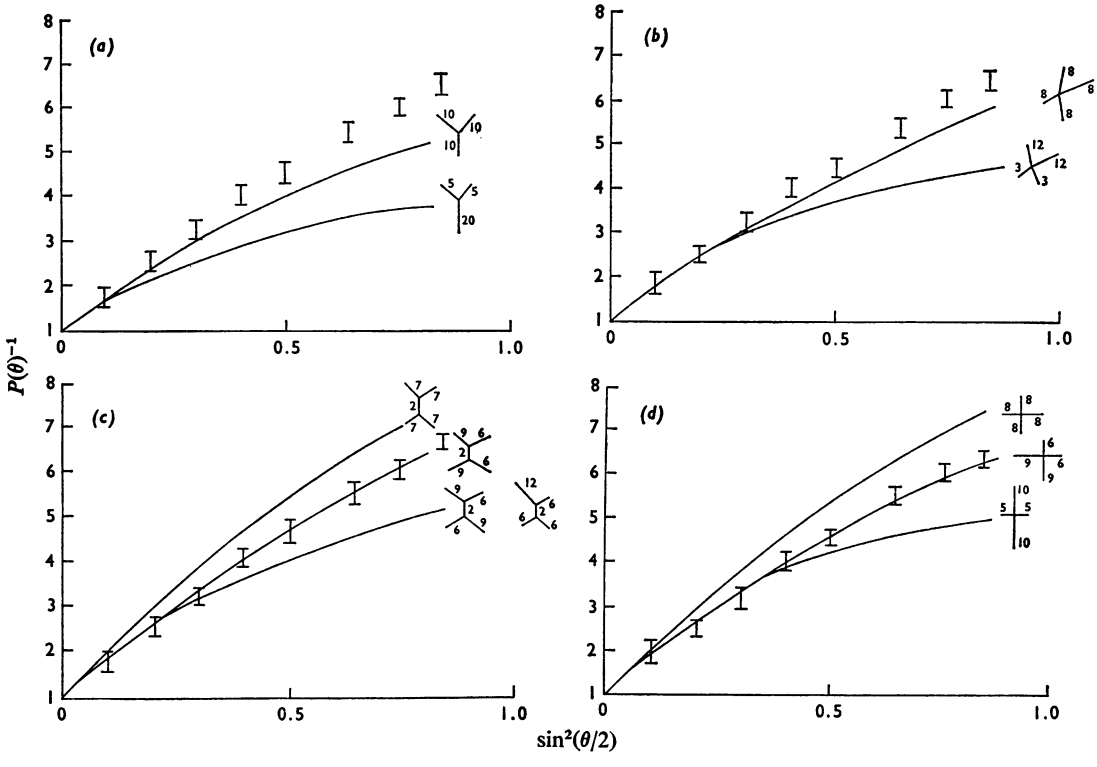

Fig. 4. *Branched structures of virus PM2 DNA*

In all cases the most symmetrical structure gives the highest theoretical curve. Introduction of asymmetry into the model leads to a lower value of the $P(\theta)^{-1}$ at each angle. The models represented are those shown in detail in Fig. 2. The numbers on the molecules represent numbers of superhelical turns of length 27 to 29nm. (*a*) Y-shaped; (*b*) tetrahedron; (*c*) double-Y-shaped; (*d*) cross-shaped.

formation in the cell the specialized secondary and tertiary structure may influence its reactivity with other macromolecules.

I thank the Science Research Council and The Royal Society for support.

References

Beard, P., Morrow, J. F., & Berg, P. (1973) *J. Virol.* **12**, 1303–1313
Botchan, P., Wang, J. C. & Echols, H. (1973) *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3077–3081
Campbell, A. M. & Eason, R. (1975) *FEBS Lett.* **55**, 212–215
Campbell, A. M. & Jolly, D. J. (1973) *Biochem. J.* **133**, 209–226
Campbell, A. M. & Lochhead, D. S. (1971) *Biochem. J.* **123**, 661–663
Crick, F. H. C. (1971) *Nature (London)* **234**, 25–27
Crick, F. H. C. & Klug, A. (1975) *Nature (London)* **255**, 530–533
Espejo, R. T. & Canelo, E. S. (1968) *Virology* **34**, 738–747
Germond, J. E., Vogt, V. M. & Hirt, B. (1974) *Eur. J. Biochem.* **43**, 591–600
Hirschman, S. Z. & Felsenfeld, G. (1966) *J. Mol. Biol.* **16**, 347–358
Hudson, B., Upholt, W. B., Devinny, J. & Vinograd, J. (1969) *Proc. Natl. Acad. Sci. U.S.A.* **62**, 813–820
Jacob, R. J., Lebowitz, J. & Kleinschmidt, A. K. (1974) *J. Virol.* **13**, 1176–1185
Jolly, D. J. & Campbell, A. M. (1972*a*) *Biochem. J.* **128**, 569–578
Jolly, D. J. & Campbell, A. M. (1972*b*) *Biochem. J.* **130**, 1019–1028
Maniatis, T., Venable, J. H. & Lerman, L. S. (1974) *J. Mol. Biol.* **84**, 37–64
Monjardino, J. & James, A. W. (1975) *Nature (London)* **255**, 249–252
Morrow, J. F. & Berg, P. (1973) *J. Virol.* **12**, 1631–1632
Pulleyblank, D. E. & Morgan, A. R. (1975) *J. Mol. Biol.* **91**, 1–13
Wang, J. C. (1974) *J. Mol. Biol.* **89**, 783–801
Waring, N. (1970) *J. Mol. Biol.* **54**, 247–279