Conformational Variation in Superhelical Deoxyribonucleic Acid

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Sedimentation experiments have shown that superhelical DNA undergoes a sharp structural transition at low ionic strength. Light-scattering experiments show that this is due to a change in conformation of the DNA rather than to a change in interactions among DNA molecules. The results show that two possible conformations can occur for superhelical DNA under routine experimental conditions and may explain the discrepancies in the number of early unwinding sites exposed by different techniques.

The basic structure adopted by small supercoiled DNA molecules in solution in the ionic-strength range 0.075 to 4.0 has been shown to be a branched conformation made up of interwound superhelical turns (Jolly & Campbell, 1972). The number and nature of the branches depends on both the sequence (Campbell & Eason, 1975) and the size (Campbell, 1976a) of the DNA and is specific for DNA molecules from each different source. The prevailing evidence suggests that the positions of the ends of the arms are dictated by the location of the early denaturing or palindrome regions in the primary structure, and it is the presence of these regions that make supercoils uniquely susceptible to reagents that react with single-stranded DNA (Jacob *et al.*, 1974; Brock *et al.*, 1975). Supercoil structure is dictated by the relative strengths of three energetically unfavourable topological restraints placed on the DNA by the inability of the double strand to unwind (Davidson, 1972). The first two are the requirement for the stiff double helix to bend along its axis and twist about its axis, and the third is for two segments of double-stranded DNA in the same molecule to lie alongside each other despite the obvious molecular repulsion. The last interaction energy is a key controlling force in supercoil structure and is responsible for the inability of superhelical DNA to take up the more tightly wound C form induced by many proteins (Campbell, 1976b). Molecules such as bacteriophage-PM2 DNA maintain tertiary structures in which the diameter of each superhelical turn is exactly equal to its segment length, so that in two-dimensional projection the structure appears as a series of jointed spheres with a diameter of 14 nm in 0.2 M-NaCl and 10 nm in 4 M-NaCl. The abolition of this branched structure and the concomitant loss of the early unwinding regions has been shown to occur at very low superhelix density (Campbell & Jolly, 1973).

Recent ultracentrifugal experiments have shown that bacteriophage-PM2 DNA exhibits unusual behaviour at low ionic strength (Vollenweider *et al.*,

1976). Whereas linear DNA molecules show a decrease in *s* value as the ionic strength is lowered, corresponding to a looser structure compatible with high intramolecular repulsion, bacteriophage-PM2 DNA molecules show a rise in *s* value over the same range, apparently indicating that a more compact structure is preferred below 1.0 M despite the increased intramolecular repulsions. In addition, electron micrographs showed that the customary branched structure of bacteriophage-PM2 DNA was absent. A single sedimentation experiment does not, however, demonstrate unequivocally that a structural change has occurred within a molecule. It could well show changes in interactions among molecules which would also affect the sedimentation rate. Light-scattering offers an excellent method of analysing molecular shape and interaction separately. The extrapolation of the Zimm plot to zero concentration affords unambiguous shape determination and the extrapolation to zero angle determines relative mass at any concentration. The work described in the present paper shows that the more compact conformation is toroidal in nature and more favoured by the molecule because of the decrease in internal repulsions in the superhelix. This structural alteration has obvious effects on the potential reactivity of superhelical DNA molecules with specific proteins and unwinding agents and may explain the discrepancies between those laboratories who differ in their estimates of the number of early unwinding sites in the supercoils.

Materials and Methods

Bacteriophage-PM2 DNA was generously given by Dr. Rosalind Cotter, Searle Research Laboratories, High Wycombe, Bucks., U.K. Light-scattering and computer curve generation have been described before (Jolly & Campbell, 1972; Campbell, 1976a,b). The refractive increment of the DNA ranged from 0.17 ± 0.003 ml/g at the lowest ionic

Fig. 1. Zimm plots of bacteriophage-PM2 DNA at ionic strengths of 0.09 (a) and 0.15 (b)

All extrapolations and calculations were as described in Campbell (1976a). K , optical constant; c , concentration in g/ml; R_θ , intensity of scattered light corrected for viewing angle. Extrapolation to zero angle gives the relative mass at any concentration and extrapolation to zero concentration gives the simple interference curve over the angular range. The condensed structure in (a) occurs at the lower salt concentration. Intermediate concentrations were not attempted.

Fig. 2. Relationship between radius of gyration (R_g) and ionic strength

The radius of gyration is obtained from the limiting slope of the Zimm plot at zero concentration. The point at high ionic strength is taken from Campbell (1976b).

Fig. 3. Theoretical structure for superhelical DNA compared with experimental points

The structures are all calculated for a molecule that has the size (5.9×10^6 daltons) and radius of gyration (73 nm) measured directly from the Zimm plot. The large radius of the toroidal molecule shown is computed from Jolly & Campbell (1972) to be 73 nm and the small radius 8 nm. $P(\theta)^{-1}$ represents the reciprocal particle-scattering factor.

strength to 0.165 M at the highest. The buffer was 10 mM-Tris/HCl/0.7 mM-EDTA, pH 7.5, with various amounts of NaCl. The number of superhelical turns remains slightly in doubt, as discussed before, and was taken to be 60. However, variation of ± 10 turns has very little effect on the molecular dimensions, and the basic conclusion of the paper is unaffected by the number of superhelical turns in this range.

Results and Discussion

Fig. 1 shows the typical Zimm plots for bacteriophage-PM2 DNA at ionic strengths of 0.09 and 0.15. The first represents a highly condensed molecule. The molecules show a tendency to interact in solution. The negative virial effects displayed by supercoils in contrast with their corresponding nicked conformation is well known and represents a lack of affinity of the molecules for solvent rather than a true affinity for each other. It has been suggested (Jolly & Campbell, 1972) that this is due to the hydrophobic bases swinging out into the medium causing unfavourable ordering of the surrounding water shell, and the slight increase in this phenomenon observed at low rather than high ionic strength reflects the increased torsional restraints in the toroidal structure. Fig. 2 shows the relationship between ionic strength and radius of gyration over the ionic-strength range tested. It is apparent that the transition in shape occurs over the same range as the transition in sedimentation coefficient. It is clear that the change in sedimentation represents a genuine conformational transition, since the radius of gyration is measured by extrapolation to zero concentration. Fig. 3 shows the computed interference curves for molecules with radii of gyration corresponding to the measured value of 73 nm. The correspondence of the data to the toroidal curve is apparent. Any form of interwound supercoil of this radius of gyration is an unrealistic proposition as the segment length of 4 nm clearly represents grossly unfavourable strand proximity.

It has often been pointed out (e.g. Crick, 1971) that the superstructural forms of biopolymers tend to adopt a helical sense that reverses that of the substructure. The left-handed toroidal form of the supercoil is therefore a natural structure to expect from right-handed DNA, if one regards the DNA molecule as a simple biopolymer. However, the unique regions in the sequence normally overcome these general rules. The opened areas at the ends of the arms allow an interwound structure, where there is minimal curvature and torsion on all other regions of the DNA. The limiting feature of this structure is the unfavourable interaction energy which dominates the refusal to take on the extra C-structure transition in high salt and clearly dictates a complete conformational transition to a torus in low salt. The dimensions of the torus are 73 nm for the large radius and 8 nm for the small one over the ionic-strength range measured, and appear to vary only marginally among these salt concentrations. This may indicate that flexibility parameters are controlling the structure

at this point (Cook & Brazell, 1975; Benyajati & Worcel, 1976). Until recently the only way these special sites could be removed was by decreasing superhelix density. However, it is now apparent that fluctuations in the ionic environment of the chromosome could control the reactivity of these sites. It can be expected that a decrease in the ionic density will lead to a greater number of reactive sites on the chromosome with much lower specificity and reactivity at any individual site as the toroidal transition occurs. This may well explain the disparity in the results of the two groups (Jacob *et al.*, 1974; Brock *et al.*, 1975) who have claimed four and eight early unwinding sites respectively for bacteriophage-PM2 DNA, since the simultaneous existence of more than one solution conformation is highly unlikely from a theoretical viewpoint (Campbell, 1976a). Reaction with unwinding proteins is frequently carried out at low ionic strength (Brock *et al.*, 1975) and may be expected to show a larger number of low-affinity sites, whereas reaction with formaldehyde in neutral buffer is carried out at high ionic strength (Jacob *et al.*, 1974) and can be expected only to expose the four high-affinity sites. The strength of the interaction energy factor makes the use of alkali as an early denaturant of the superhelical material likely to yield variable results, as a toroidal conformation will become greatly favoured under such conditions.

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