*TWISTED CIRCULAR DNA: SEDIMENTATION COEFFICIENTS AND THE NUMBER OF TWISTS**

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Recently, Vinograd *et al.*[1] have proposed that most of the DNA from polyoma virus consists of twisted, circular, double-stranded molecules which have a sedimentation coefficient of 20*S*. One single-strand scission in this DNA produces another form of circular DNA with $S^{\circ}_{20,w} = 16S$. Similar interconversions between two apparently circular forms have been noted in DNA from several other viruses: SV40 virus by Crawford and Black,[2] the replicating form of ϕX174 by Jansz and Pouwels,[3] and papilloma virus by Crawford.[4]

In this communication we present a calculation of the ratio of the sedimentation coefficients of the twisted and untwisted circular forms, as a function of the number of twists. This calculation has two motivations. The first is to show that the experimental ratio of 1.25 or somewhat higher is consistent with the identification of the faster-sedimenting form as a twisted circular molecule. The second is to obtain an estimate of the number of twists. This number is difficult to estimate from electron micrographs[1] because of possible strand breaks during grid preparation and the uncertain action of surface spreading forces.

Calculation of Sedimentation Coefficients.—We shall use the following model for the structure of twisted circular DNA. A circular molecule containing t twists or left-handed tertiary turns[1] has $2t$ strand crossovers. In our model it will be assumed that the crossover points are equally spaced and fixed, dividing the molecule into $l(= 2t + 1)$ equal-sized loops. Each loop contains N frictional elements contributing to resistance to flow in a sedimenting field; thus the entire molecule contains Nl frictional elements.

The translational diffusion coefficient D of a polymer containing n frictional elements, each with frictional coefficient ξ, is given by Kirkwood[5] as

$$D = (kT/n\xi)[1 + (\xi/6\pi\eta n) \sum_{\substack{i=0 \\ i\neq j}}^{n} \sum_{j=0}^{n} \langle R_{ij}^{-1} \rangle]. \tag{1}$$

η is the viscosity of the solvent, and $\langle R_{ij}^{-1} \rangle$ is the reciprocal distance between frictional elements i and j, averaged over all internal configurations of the polymer chain.

The sedimentation coefficient S can be calculated from (1) and the familiar relation

$$S = DM(1 - \bar{v}\rho)/RT. \tag{2}$$

Apart from the restriction of fixed crossover points, the twisted circular DNA is assumed to behave like a flexible, random-walking polymer. Because of excluded volume effects, chain stiffness, and polyelectrolyte effects, the average dimensions of DNA molecules are somewhat larger than those predicted for a Gaussian, freely jointed chain. This chain expansion may be represented by a parameter ϵ, such

that if b is the distance between adjacent frictional elements, the mean square distance between elements i and j in a linear chain is

$$\langle R_{ij}^2 \rangle = b^2 |i - j|^{1+\epsilon}, \tag{3}$$

where $\epsilon = 0$ corresponds to an ideal Gaussian chain.

We may divide the double sum in (1) into contributions from hydrodynamic interaction between elements in the same loop and between those in different loops. The mean square distance between elements i and j in the same loop is[6]

$$\langle R_{ij}^2 \rangle = b^2 N^{1+\epsilon} \left|\frac{i-j}{N}\right|^{1+\epsilon} \left(1 - \left|\frac{i-j}{N}\right|\right)^{1+\epsilon} \div \left[\left|\frac{i-j}{N}\right|^{1+\epsilon} + \left(1 - \left|\frac{i-j}{N}\right|\right)^{1+\epsilon}\right], \tag{4}$$

while that between elements i and j in loops separated by k other loops is

$$\langle R_{ij}^2 \rangle = b^2 N^{1+\epsilon} \left[\frac{\left(\frac{i}{N}\right)^{1+\epsilon}\left(1 - \frac{i}{N}\right)^{1+\epsilon}}{\left(\frac{i}{N}\right)^{1+\epsilon} + \left(1 - \frac{i}{N}\right)^{1+\epsilon}} + \frac{\left(\frac{j}{N}\right)^{1+\epsilon}\left(1 - \frac{j}{N}\right)^{1+\epsilon}}{\left(\frac{j}{N}\right)^{1+\epsilon} + \left(1 - \frac{j}{N}\right)^{1+\epsilon}} + \left(\frac{1}{2}\right)^{2+\epsilon} k\right]. \tag{5}$$

In these equations i and j can run between 0 and N, while k goes between 0 and l-2.

The mean reciprocal distance is related to the mean square distance by the expression

$$\langle R_{ij}^{-1} \rangle = (6/\pi)^{1/2} \langle R_{ij}^2 \rangle^{-1/2}. \tag{6}$$

Substitution of (4)–(6) into (1), conversion of sums to integrals, and use of (2) eventually leads to

$$S_l/S_1 = l^{-(1-\epsilon)/2} \left\{1 + 4\left[\sum_{k=0}^{l-2} (l - k - 1) I_k(\epsilon)\right] \Big/ l I'(\epsilon)\right\}, \tag{7}$$

where S_l is the sedimentation coefficient of the chain containing l loops, and $l = 1$ denotes the untwisted chain. The integrals $I_k(\epsilon)$ and $I'(\epsilon)$ are defined as

$$I_k(\epsilon) = \int_0^{1/2}\int_0^{1/2} \left[\frac{r^{1+\epsilon}(1-r)^{1+\epsilon}}{r^{1+\epsilon} + (1-r)^{1+\epsilon}} + \frac{s^{1+\epsilon}(1-s)^{1+\epsilon}}{s^{1+\epsilon} + (1-s)^{1+\epsilon}} + \left(\frac{1}{2}\right)^{2+\epsilon} k\right]^{-1/2} dr ds, \tag{8}$$

$$I'(\epsilon) = \int_0^1 q^{-(1+\epsilon)/2} (1-q)^{(1-\epsilon)/2} \left[q^{1+\epsilon} + (1-q)^{1+\epsilon}\right]^{1/2} dq. \tag{9}$$

Equation (7) assumes that the term containing the double sum in (1) is much greater than unity. This "nondraining" assumption is certainly justified for the high-molecular-weight polymers considered here.

Results.—The value of ϵ appropriate to double-stranded DNA of a given molecular weight may be calculated from the relations[6]

$$\frac{dlnS}{dlnM} \approx \frac{1-\epsilon}{2}, \qquad \frac{dln[\eta]}{dlnM} \approx \frac{1+3\epsilon}{2} \tag{10}$$

and the empirical equations for S and $[\eta]$ of DNA as functions of molecular weight M given by Crothers and Zimm.[7] The values of ϵ calculated from S and from $[\eta]$ differ slightly, but not enough to affect our results significantly. All the DNA's mentioned above, except that from papilloma virus, have a molecular weight of about 3×10^6; for these, $\epsilon = 0.25 \pm 0.04$. Papilloma virus DNA has a molecular weight of about 5.3×10^6; for this, $\epsilon = 0.21 \pm 0.04$.

Integrals (8) and (9) have been evaluated numerically for these and other values of ϵ and the results have been substituted into (7). In Table 1 are given the sedimentation coefficient ratios calculated from (7) for numbers of loops between 1 and 10, with $\epsilon = 0.21$ and 0.25.

Plots of the sedimentation coefficient ratio versus log l are nearly linear. An approximate numerical expression which has been found to be quite accurate for l between 2 and 10 and ϵ between 0.11 and 0.25 is

$$(S_l/S_1) - 1 = 0.01 - 0.11\,\epsilon + (0.39 + 0.71\,\epsilon)\log_{10} l. \tag{11}$$

It is evident from this expression that S_l/S_1 is only weakly dependent on ϵ for small values of l.

Discussion.—The values of the sedimentation coefficients, and their ratios, of the presumed twisted and untwisted circular forms of the viral DNA's mentioned above are as follows: polyoma, 20/16 = 1.25; SV40, 21.2/16.1 = 1.31; ϕX174 RF, 20.7/16.0 = 1.29; and papilloma, 28.2/20.2 = 1.40. We note immediately that these ratios fall in the range for moderate values of l given in Table 1. Hence, the identification of the two forms as twisted and untwisted circles seems plausible in all cases.

TABLE 1

SEDIMENTATION COEFFICIENT RATIOS FOR CIRCULAR DNA WITH l LOOPS

l	S_l/S_1, $\epsilon = 0.21$	S_l/S_1, $\epsilon = 0.25$
1	1.000	1.000
2	1.148	1.151
3	1.240	1.247
4	1.308	1.318
5	1.362	1.375
6	1.406	1.422
7	1.444	1.463
8	1.478	1.498
9	1.507	1.530
10	1.533	1.559

Further, comparison of Table 1 and the experimental ratios suggests that polyoma DNA contains three loops or one twist; ϕX and SV40 DNA's contain about four loops or 1.5 twists; and papilloma DNA contains six loops or 2.5 twists. These values are in the range observed from electron micrographs. It is interesting to note that although papilloma virus DNA apparently has more twists than the other three, it is also almost twice as large, so that the number of twists per unit length appears roughly constant in all four of these DNA's.

Finally, we may attempt to assess the effect of some of the approximations in the sedimentation coefficient calculation on the estimation of the number of loops. A first approximation is that the strands touch each other at the crossover points, while it is likely that in fact they are kept somewhat apart by repulsive interactions. This approximation would overestimate $\langle R_{ij}^{-1} \rangle$ between elements near the crossover points, thereby raising the calculated S_l/S_1 and lowering the estimated value of l for a given ratio. On the other hand, the assumption of equal-sized loops would tend

to underestimate $\langle R_{ij}^{-1} \rangle$ between elements in loops that are smaller than average, more than it would overestimate $\langle R_{ij}^{-1} \rangle$ in loops larger than average, and thus would lower S_l/S_1. Thus, the effects of these two approximations will cancel to some extent.

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PURINE BINDING TO DINUCLEOTIDES: EVIDENCE FOR BASE STACKING AND INSERTION

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In an effort to understand the factors which may contribute to the stabilization of nucleic acids as well as the basic mechanism of the recognition process involved in the enzymatic replication of nucleic acids *in vitro*, we have embarked upon a systematic study of the interaction of biological bases and nucleosides with simple oligonucleotides by proton magnetic resonance. We wish to report here results which we have obtained on the interaction of purine with the following 3′ → 5′-dinucleotides: TpT, TpdU, and dUpT (T = thymidine, dU = 2′-deoxyuridine).

All the spectra reported in this communication were taken on a Varian A-60 NMR spectrometer with probe temperature at 30°C. The dinucleotides were dissolved in D_2O in the form of the ammonium salt.

The proton magnetic resonance spectrum of TpT in the regions of the thymine CH_3 and H_6 protons and the H_1' protons of the sugar moieties is shown in Figure 1*a*. For comparison, the same spectral regions for the thymidine nucleoside are also given (Fig. 1*b*). The protons of the two bases of TpT and the base protons in thymidine are, apparently, magnetically indistinguishable. The H_6 proton resonance in TpT is noticeably broader than that in thymidine, thereby suggesting that the two H_6 protons in TpT may not be exactly magnetically equivalent. The two H_1' protons of the dinucleotide are clearly not equivalent, and the resonance spectrum for these protons consists of a superposition of two 1:2:1 triplets. Evidently, the asymmetric attachment of the PO_4^- to the sugar moieties affects the magnetic environments at these protons differently. The chemical shift between the two H_1' protons is about 6 cps and is roughly equal to the spin-spin splittings