Light-Scattering Studies on Deoxyribonucleic Acid Flexibility

THE SOLUTION PROPERTIES OF A SMALL CIRCULAR DEOXYRIBONUCLEIC ACID MOLECULE

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Previous investigations on the persistence length of DNA in solution have revealed large discrepancies between hydrodynamic results and those from light-scattering techniques which have potentially a greater resolving power. The information obtained from experiments on a small circular DNA molecule has resolved these discrepancies. The non-superhelical circular double-stranded DNA molecule from bacteriophage ϕ X174-infected cells is small enough to permit accurate light-scattering extrapolations, and its solutions have negligible anisotropy. The persistence length obtained from experimental investigations on this molecule is comparable with that obtained by hydrodynamic techniques, even with variation of the excluded-volume factor.

Two major parameters are used by convention to describe the longitudinal stiffness of DNA molecules in solution. These are the Kuhn statistical segment length (Kuhn, 1936, 1939) and the persistence length (Kratky & Porod, 1949; Flory, 1969). Since the former has twice the numerical value of the latter the two are readily interconvertible. A quantitative assessment of this factor is an integral part of any thermodynamic calculations involving DNA packaging and unwinding, speed and modes of replication and transcription, and the interaction of DNA with other macromolecules (see, for example, Gray & Hearst, 1968). ϕ X174 RF II DNA* is a most suitable molecule for the unequivocal determination of such a factor because of its small size (3.2×10^6 daltons) and its isotropy in solution. Previous light-scattering investigations have been conducted on molecules which are either too large, or which lack homogeneity and isotropy and consequently the results have differed from both the hydrodynamic results and the results presented in this paper.

Recent theories of the solution behaviour of DNA have treated it as a worm-like coil (Kratky & Porod, 1949) and have characterized it by the molecular weight, M , the excluded-volume parameter, ϵ (Bloomfield & Zimm, 1966; Peterlin, 1955), the persistence length, $1/2\lambda$, and the hydrodynamic diameter of the double helix, d . The last three are assumed to be independent of the source of the DNA and to depend only on the solution conditions. Results from sedimentation and viscosity measurements (Gray *et al.*, 1967; Sharp & Bloomfield,

1968*a,b*; Ullman, 1968; Hearst *et al.*, 1968*a,b*; Hays *et al.*, 1969; Triebel *et al.*, 1971) show that at 25°C, neutral pH and $1/0.2$ the values of $\epsilon = 0.11$, $1/2\lambda = 45$ nm and $d = 2.7$ nm are fairly well established for native linear DNA.

The technique of light-scattering has potentially much greater resolving power than hydrodynamic methods because it provides a direct measure of the root-mean-square radius of the molecule and of the molecular weight and further because a simple interference curve can be predicted for various molecular conformations and compared with experimental values. However, it has been accepted for several years that for large DNA molecules (greater than 6×10^6 daltons) extrapolation of results from 30° to 0° yields values of molecular weight approximately one-half the actual value (Froelich *et al.*, 1963). The persistence-length values have varied from 40 nm to 350 nm (Geiduschek & Holtzer, 1958; Sharp & Bloomfield, 1968*b*; Hays *et al.*, 1969; Eisenberg, 1969; Jennings & Plummer, 1970). However, even with low-angle instruments (down to 10°) with large homogeneous DNA molecules such as bacteriophage T7 DNA ($M = 25 \times 10^6$) (Harpst *et al.*, 1968) the results were inaccurate as extrapolation to zero angle was still too large and experiments at lower angle become necessary. Schmid *et al.* (1971) and Zimm (1948) have estimated that extrapolation to 0° is satisfactory from values of $P(\theta)^{-1}$ less than or equal to 1.3, or $[P(\theta)^{-1}]^{1/2}$ less than or equal to 1.3 for a Berry (1966) plot, where $P(\theta)$ is the particle-scattering factor which has a value of 1.0 at 0°.

A second factor other than size which has complicated light-scattering investigations is anisotropy effects which arise with small rod-like DNA molecules (Weill *et al.*, 1968). Thus the use of highly sonicated DNA molecules examined at angles of 30° and

* Abbreviations: ϕ X174 RF I, intact (un-nicked) circular duplex intracellular replicative form of bacteriophage ϕ X174 DNA; ϕ X174 RF II, nicked circular duplex intracellular replicative form of bacteriophage ϕ X174 DNA.

greater does not lead to a correct measure of persistence length unless this is allowed for (Schmid *et al.*, 1971). Cohen & Eisenberg (1966) have been criticized for neglecting this effect.

ϕ X174 RF II DNA is both small and circular and the two problems can therefore be avoided by use of this molecule. Anisotropy is negligible and meaningful extrapolations to 0° can be made. Comparison of the experimental results with the predicted scattering envelopes for the persistence length and the excluded-volume parameter of various values can therefore be made and a reliable measure of DNA stiffness can be obtained.

There have been two previous published investigations on the light-scattering properties of circular DNA. Dawson & Harpst (1971) have studied circular bacteriophage λ DNA which has the disadvantage of being too large for conventional instruments ($M = 32 \times 10^6$) so that the extrapolation to zero angle is not reliable. No attempt was made to estimate the persistence length. DNA molecules from this source are frequently contaminated with linear or aggregated forms of DNA because of the cohesive ends of the molecule. Jolly & Campbell (1972) have studied the superhelical form of double-stranded ϕ X174 DNA which has a completely different solution structure owing to the tertiary loops in the molecule.

In the experiments reported here we have been able to show that the persistence length obtained from meaningful light-scattering experiments does agree with that which has been obtained from hydrodynamic results and that the hydrodynamic value is therefore the one that should be employed in calculations involving DNA packaging and unwinding.

Materials and Methods

Preparation and analysis of DNA

The replicative double-stranded form of bacteriophage ϕ X174 DNA was prepared and the two forms were separated as described previously (Jolly & Campbell, 1972). All light-scattering experiments were performed under nuclease-free conditions with sterilized glassware. The DNA was analysed in the ultracentrifuge before and after the scattering experiments by band sedimentation through 2.8M-CsCl, pH7. The $s_{20,w}$ value was 17S.

Light-scattering measurements

All light-scattering experiments were carried out in a Fica 50 photogonioidiffusometer as described by Jolly & Campbell (1972) and solutions were clarified

by filtration with the usual precautions. Concentration measurements were made in the same manner as before (Jolly & Campbell, 1972) and the refractive-index increment of DNA was taken as 0.166ml/g (Krasna, 1970). Measurements to detect anisotropy were made by measuring the depolarization of scattered light (Geiduschek & Holtzer, 1958) from incident unpolarized light by using polarization filters supplied with the instrument.

Buffer

The buffer employed in these experiments was BPES (6mM- Na_2HPO_4 , 2mM- NaH_2PO_4 , 1mM-dissodium EDTA and 0.179M-NaCl, pH6.8).

Analysis of data

The light-scattering curves for all the conformations were processed on a PDP 8/L digital computer.

Results

Theory

For any macromolecule examined by light-scattering the variation of scattered intensity with the scattering angle, θ , is expressed by a function $P(\theta)$ (Geiduschek & Holtzer, 1958). Generally:

$$P(\theta) = \frac{1}{N^2} \int_0^\infty f(r) \frac{\sin \mu r}{\mu r} dr \quad (1)$$

where $\mu = 4\pi \sin(\theta/2)/\bar{\lambda}$, $P(\theta)$ = scattered intensity at θ /scattered intensity at 0, $\bar{\lambda}$ = wavelength of light in solution, N = number of segments in molecule and $f(r)$ = average number of pairs of elements separated by a distance r

$$= N \int_0^N w(r, t) dt \text{ for a circular molecule}$$

where $w(r, t)$ is the probability that elements separated by a distance t along the contour length of the molecule are separated by a distance r in space.

Hence:

$$P(\theta) = \frac{1}{N} \int_0^N \int_0^\infty w(r, t) \frac{\sin \mu r}{\mu r} dr dt \quad (2)$$

For $w(r, t)$ the Daniels distribution function (Daniels, 1952) has been used with the modification to include the excluded-volume parameter (Gray *et al.*, 1967; Sharp & Bloomfield, 1968b). This gives:

$$w(r, t) dr = \left[\frac{3\lambda}{\pi t} \frac{1}{(\lambda t)^3} \right]^{\frac{1}{2}} \left(\frac{2}{\pi} \right)^{\frac{1}{2}} \exp \left[-\frac{3\lambda}{2t} \frac{1}{(\lambda t)^3} r^2 \right] \times \left[1 - \frac{5}{8\lambda t} + \frac{2}{t^2} \frac{1}{(\lambda t)^3} r^2 - \frac{33\lambda}{40t^3} \frac{1}{(\lambda t)^2} r^4 \right] r^2 dr \quad (3)$$

where ϵ is the excluded-volume factor, $1/2\lambda$ is the persistence length and all distances are measured in terms of b , an arbitrary segment length.

This equation for the distribution function of a linear molecule is easily converted into that of a circular molecule following Zimm & Stockmayer (1949).

Hence for a circular molecule:

$$w(r, t) dr = A \exp \left\{ \frac{-3\lambda}{2} \left[\frac{(N-t)^{(1+\epsilon)} + t^{(1+\epsilon)}}{(N-t)^{(1+\epsilon)} t^{(1+\epsilon)} \lambda^\epsilon} \right] r^2 \right\} \times \left[1 - \frac{5}{8\lambda t} + \frac{2r^2}{t^{(2+\epsilon)} \lambda^\epsilon} - \frac{33r^4 \lambda^{(1-2\epsilon)}}{40t^{(3-2\epsilon)}} \right] \times \left[1 - \frac{5}{8\lambda(N-t)} + \frac{2r^2}{(N-t)^{(2+\epsilon)} \lambda^\epsilon} - \frac{33r^4 \lambda^{(1-2\epsilon)}}{40(N-t)^{(3-2\epsilon)}} \right] r^2 dr \quad (4)$$

where A is the normalizing constant, and

$$1/A = \int_0^\infty \exp(-kr^2)(a+br^2+cr^4)(p+qr^2+vr^4)r^2 dr$$

where

$$a = 1 - \frac{5}{8\lambda t} \quad b = \frac{2}{t^2} \frac{1}{(\lambda t)^\epsilon}$$

Substituting this in eqn. (2), when N is large, gives:

$$P(\theta) = \frac{1}{N} \int_0^N Z dt = \frac{1}{N} + \frac{1}{N} \sum_{t=1}^N (Z) \quad (6)$$

However at small values of t up to x (the segment number where the theoretical distribution functions

intersect), the Daniels distribution function is not correct for worm-like coils and must be replaced by the expression derived from the exact distribution function (Hearst & Stockmayer, 1962; Gray *et al.*, 1967; Sharp & Bloomfield, 1968b).

Hence a more accurate description than eqn. (2) is:

$$P(\theta) = \frac{1}{N} \int_0^x \int_0^\infty [W(r, t) - w(r, t)] \frac{\sin \mu r}{\mu r} dr dt + \frac{1}{N} \int_0^N \int_0^\infty w(r, t) \frac{\sin \mu r}{\mu r} dr dt \quad (7)$$

$$c = -\frac{33\lambda}{40t^3} \frac{1}{(\lambda t)^{2\epsilon}} \quad p = 1 - \frac{5}{8\lambda(N-t)}$$

$$q = \frac{2}{(N-t)^2} \frac{1}{[\lambda(N-t)]^\epsilon} \quad v = -\frac{33\lambda}{40(N-t)^3} \frac{1}{[\lambda(N-t)]^{2\epsilon}}$$

and

$$k = \frac{3\lambda}{2} \left\{ \frac{(N-t)[\lambda(N-t)]^\epsilon + t(\lambda t)^\epsilon}{t(N-t)(\lambda t)^\epsilon(N-t)^\epsilon \lambda^\epsilon} \right\}$$

Thus:

$$\int_0^\infty w(r, t) \frac{\sin \mu r}{\mu r} dr = Z$$

$$= A \left(\frac{\pi}{k} \right)^{\frac{1}{2}} \frac{1}{4k} e^{-\mu^2/4k} \left[ap + (aq + pb) \left(3 - \frac{\mu^2}{2k} \right) / 2k + (av + bq + cp) \left(15 - \frac{5\mu^2}{k} + \frac{\mu^4}{4k^2} \right) / 4k^2 \right. \\ \left. + (bv + cq) \left(105 - \frac{105\mu^2}{2k} + \frac{2\mu^4}{4k^2} - \frac{\mu^6}{8k^3} \right) / 8k^3 + cv \left(\frac{945}{2} - \frac{315\mu^2}{k} + \frac{189\mu^4}{4k^2} - \frac{9\mu^6}{4k^3} + \frac{\mu^8}{32k^4} \right) / 8k^4 \right] \quad (5)$$

where $w(r, t)$ is the Daniels-type distribution function and $W(r, t)$ is the real distribution function.

Intersection between the two distributions occurs at values of $t \ll N$ so that the effect of circularity on the distribution should be negligible and straight-chain expressions for $W(r, t)$ can be used (Gray *et al.*, 1967).

Thus the model of Kratky & Porod (1949) without excluded volume can be used at small values of t since at these intersegment distances the excluded-volume factor is negligible and can be ignored up to $t = x$, where intersection of the distribution occurs.

Thus:

$$\begin{aligned}
 P(\theta) &= \frac{1}{N} \sum_{t=1}^x \left[\int_0^{\infty} W(r, t) \frac{\sin \mu r}{\mu r} dr \right] - \frac{1}{N} \sum_{t=1}^x \left[\int_0^{\infty} w(r, t) \frac{\sin \mu r}{\mu r} dr \right] \\
 &\quad + \frac{1}{N} \sum_{t=1}^N \left[\int_0^{\infty} w(r, t) \frac{\sin \mu r}{\mu r} dr \right] + \frac{1}{N} \\
 &= \frac{1}{N} \sum_{t=1}^x \left[\int_0^{\infty} W(r, t) \frac{\sin \mu r}{\mu r} dr \right] + \frac{1}{N} \sum_{t=x+1}^N (Z) + \frac{1}{N}
 \end{aligned} \tag{8}$$

$$\int_0^{\infty} W(r, t) \frac{\sin \mu r}{\mu r} dr = 1 - \frac{\mu^2 \langle r^2 \rangle}{3!} + \frac{\mu^4 \langle r^4 \rangle}{5!} \tag{9}$$

where

$$\langle r^n \rangle = \int_0^{\infty} r^n W(r, t) dr$$

As we are dealing with small values of r , higher terms in eqn. (9) may be neglected.

Since $\langle r^2 \rangle$ and $\langle r^4 \rangle$ have been calculated (Hermans & Ullman, 1952) the first term in eqn. (8) can be evaluated. The intersection point at $t = x$ of the two distributions is taken as the point where $\langle r^2 \rangle$ is equal when calculated from both distributions. The validity of this procedure has been checked by evaluating $\langle r^2 \rangle$ at $t = x$ according to the equations of Sharp & Bloomfield (1968b) and, to ensure similar values, comparing this with the value from our expression and that of Hermans & Ullman (1952). The two values should be and are similar.

The total summation in eqn. (8) can therefore be carried out by computer, since an analytical solution does not seem possible for assigned values of N , λ , ϵ , μ , x and segment size.

Experimental

No anisotropy was detectable in solutions of ϕ X174 RF II DNA, after solvent subtraction. At 90° , no horizontally polarized light was detectable, and on extrapolation to 0° , the ratio of the vertically to horizontally polarized components became 1 as expected.

Three different experiments, on different days, with different samples were performed. The results of these were totally compatible. Top and bottom concentrations were about 45 and $19 \mu\text{g/ml}$ respectively.

The results were initially plotted as Zimm (1948)

plots (Fig. 1). As can be seen, due to curvature of the plot, the extrapolation to zero angle relies on the two lowest-angle points alone. Plotting the data by the method of Berry (1966) does not help in this case.

To check this extrapolation, therefore, in one experiment a large number of low-angle readings were made (seven between 37.5° and 26°). These were plotted in a Berry plot (Fig. 2a) and a Zimm plot (Fig. 2b) where $[Kc/R(\theta)]^{\frac{1}{2}}$, not $Kc/R(\theta)$, is plotted on the ordinate, to attempt to eliminate any curvature at low angles. Fig. 2 shows that, in both plots, extrapolations by using only points at 30° and 37.5° are fully vindicated by experimental points at intermediate and slightly lower angles. As in Fig. 1, there is clearly a change in direction of the curves for the portions above and below approximately 37.5° .

The full Zimm plots (30° – 150° , with points at 30° , 37.5° and above) gave a molecular weight of $3.35 \pm 0.2 \times 10^6$; both the Zimm and Berry plots of low-angle data gave a molecular weight of $3.18 \pm 0.2 \times 10^6$. These values agree well with the value of $3.17 \pm 0.19 \times 10^6$ obtained previously for RF I DNA (Jolly & Campbell, 1972).

The full Zimm plots gave a root-mean-square radius (R_g) of $109.5 \pm 15 \text{ nm}$; from the low-angle Zimm and Berry plots the values were $111.0 \pm 11 \text{ nm}$ and $109.0 \pm 11 \text{ nm}$. These all agree well, again vindicating the extrapolation in Fig. 1; the errors are estimated from least-good slopes. These values are surprisingly near the value for supercoiled RF I, which was previously found to be $103.5 \pm 12 \text{ nm}$ (Jolly & Campbell, 1972); this latter value is now thought, from later experiments (D. J. Jolly & A. M. Campbell, unpublished work), to be slightly lower, about $95 \pm 12 \text{ nm}$.

For the experimental curve of $P(\theta)^{-1}$ against $\sin^2(\theta/2)$, the values of $[KC/R(\theta)]_{\epsilon=0}$ at finite angles

Fig. 1. Zimm plot for ϕ X174 RF II DNA in BPES buffer at 25°C

The scattering angle, θ , varied from 30° to 150°. On the abscissa the concentration (c) is in mg/ml; otherwise all units are in the c.g.s. system. The formula $[Kc/R\theta]_{c=0} = 1/M$ normally allows estimation of the molecular weight, M , where $K = 2\pi^2 n^2 (dn/dc)^2 / N\bar{\lambda}^4$ is a constant for these experiments (1.807×10^{-7}), and $R(\theta) = r^2 i_\theta / I_0 (1 + \cos^2 \theta)$. Here n is the refractive index of the solvent, dn/dc the refractive-index increment, N Avogadro's number, $\bar{\lambda}$ the wavelength of light *in vacuo*, i_θ the intensity of scattered light at θ , I_0 the intensity of incident light, both per unit volume, and r is the distance of the receiver from the scattering point. The formulae $[Kc/R(\theta)]_{c=0} (1/M) [1/P(\theta)] = (1/M) [1 + 16\pi^2 Rg^2 \sin^2(\theta/2) / 3(\bar{\lambda}/n)^2 + \dots]$ and $[Kc/R(\theta)]_{\theta=0} = 1/M + 2Bc + \dots$ allow estimation of the root-mean-square radius, Rg , and second virial coefficient, B , respectively. Here B is approx. zero.

were taken from the full Zimm plots but the value at $\theta = 0^\circ$ was taken from the multi-point low-angle extrapolation.

For the theoretical curves, the parameters used were: number of segments, N , 547; segment size, 3.0 nm; persistence length from 30–70 nm. The excluded-volume factor, ϵ , was used at two values; these were 0.11, the value accepted for linear DNA, and 0.141, the corresponding value for a circular DNA of molecular weight 3.2×10^6 (Gray *et al.*, 1967). The value of x , the segment number where the theoretical distribution functions intersect, varied with persistence length and the excluded-volume

factor, as shown in Table 1, as are the values of $\langle r_x^2 \rangle$ for the intersection of the Kratky & Porod (1949) distribution with ours. Also shown, for comparison, is the value of $\langle r^2 \rangle$, obtained by using the same parameters, for the distribution of Sharp & Bloomfield (1968b) for linear DNA.

The comparisons of experimental with theoretical results are shown in Figs. 3 and 4, for $\epsilon = 0.141$ and 0.11 respectively. As can be seen this makes only a small difference to the theoretical curves, although the former is more appropriate. By visual examination, at $\epsilon = 0.141$, the experimental points fit the theoretical curves well for a value of the persistence length around or just over 40 nm. We estimate a value of 41.0 ± 3.5 nm for this parameter. For $\epsilon = 0.11$, there is no great difference and the persistence length appears only marginally higher. Thus, even if the value of ϵ is not certain, the error in the persistence length is not increased much.

It is also worth noting that the general form of the experimental and theoretical curves agrees with that predicted for circular flexible chains by Casassa (1965).

Discussion

Molecular weight

The molecular weight found agrees well with the value for RF I previously found (Jolly & Campbell, 1972) of $3.17 \times 10^6 \pm 0.19 \times 10^6$. This value has since been confirmed in a number of experiments under different conditions (D. J. Jolly & A. M. Campbell, unpublished work). The slightly higher value of molecular weight in the absence of a large number of low-angle points as opposed to the case when these are available, and the slightly better agreement with the values of the molecular weight of RF I suggest that even when no lower angles are examined than normal, a large number of points near the lowest angle lead to a more-convincing extrapolation to zero angle, since this gives a number of values of $P(\theta)^{-1}$ under 1.3 for correct extrapolation, as stipulated by Schmid *et al.* (1971). The curvature just above 30° for RF II DNA indicates that these molecules approach the limit of dimensions which can be examined by light-scattering down to 30°. Thus linear DNA molecules of molecular weight 3×10^6 are probably still slightly too large for this method, as noted by Jennings & Plummer (1970), although these workers used shorter-wavelength light (436 nm). Recent surveys of molecular-weight determinations (Freifelder, 1970; Schmid & Hearst, 1969) on whole native DNA molecules have indicated that previously accepted values of molecular weights (Thomas & MacHattie, 1967) were somewhat high, in particular for bacteriophage λ and T4 DNA. These

Fig. 2. Low-angle data for extrapolation to 0°

All units are in c.g.s. system; the temperature was $25 \pm 0.2^\circ\text{C}$ and the buffer was BPES. The concentration range was approx. $30\text{--}65\ \mu\text{g/ml}$, and all symbols are as in the legend to Fig. 1. The numbers on the right-hand side of both plots are the scattering angles, in degrees, for that set of points. (a) Data plotted as a Berry (1966) plot; information may be extracted as follows: $\{[Kc/R(\theta)]^{\frac{1}{2}}\}_{c=0} = (1/M)^{\frac{1}{2}}$ gives the molecular weight; $\{[Kc/R(\theta)]^{\frac{1}{2}}\}_{c=0} = (1/M)^{\frac{1}{2}} [1/P(\theta)]^{\frac{1}{2}} = (1/M)^{\frac{1}{2}} [1 + 8\pi^2 Rg^2 \sin^2(\theta/2)/3(\lambda/n)^2 + \dots]_{\theta=0}$ gives the root-mean-square radius, Rg . (b) Data plotted as a Zimm (1948) plot; information may be extracted as in the legend to Fig. 1.

and other workers (see Freifelder, 1970, for a review) have started to establish soundly based values for DNA molecular weights by absolute methods, e.g., sedimentation equilibrium in a density gradient, to allow their use as 'standards' in relative methods, e.g. electron microscopy, sedimentation velocity. For good reasons (Schmid *et al.*, 1971) light-scattering results have been regarded as unreliable. However, for the reasons listed above and because further examination of our results has shown a compatibility of worm-like-coil properties obtained from light-scattering with those from viscosity and sedimentation, not previously achieved, we feel our value for the molecular weight for ϕX174 RF DNA is quite

accurate and therefore usable as a standard, with, if necessary, allowances for circularity.

Sinsheimer (1959) obtained from light-scattering on single-stranded ϕX174 DNA a molecular weight of 1.7×10^6 and hence $3.4 \times 10^6 \pm 0.2 \times 10^6$ for the double-stranded form. As pointed out by Schmid & Hearst (1969), the value of the refractive index increment (dn/dc) used now seems incorrect and therefore the results are questionable; reinterpretation in view of more recent values for dn/dc (Cohen & Eisenberg, 1968; Krasna, 1970) indicates a molecular weight of 4.5×10^6 . We presume that the difference between this and our results is due mainly to modern methods of dust removal.

Table 1. Variation of the segment numbers where intersection of the two distribution functions occur with variation in the parameters, ϵ and persistence length

$\langle r^2 \rangle_x$ is the mean square separation of two segments x apart on the polymer chain. Values (a) are those which are the same for the Kratky & Porod (1949) distribution and the circular distribution calculated in the present paper. Values (b) are computed from the distribution of Sharp & Bloomfield (1968a) for linear worm-like coils for the same parameters as (a). At least for the lower values of the persistence length, values (a) and (b) are very similar, showing circularity has little effect at small intersegment numbers. The segment size is 3.0 nm. The equations used for $\langle r^2 \rangle_t$ at $t = x$ were as follows:

Kratky & Porod (1949) distribution,

$$\langle r^2 \rangle_t = t/\lambda - (1 - \exp[2\lambda t])/2\lambda^2$$

Jolly & Campbell (circular molecule, the present paper),

$$\langle r^2 \rangle_t = 3[ap + 5(aq + bp)/2k + 35(av + bq + cp)/4k^2 + 315(bv + cq)/8k^3 + 3465cv/16k^4]/2kZ$$

Sharp & Bloomfield (1968b) distribution,

$$\langle r^2 \rangle_t = t^{1+s}(1 - 1/2\lambda t)/\lambda^{1-s}$$

The first two equations were used to calculate, by trial and error, values of $\langle r^2 \rangle_t$ which were the same for the two distributions, where $t = x$. These are given in (a). The third equation was used to calculate the values in columns (b) by using the same parameters as in the second equation.

$\epsilon = 0.141$				$\epsilon = 0.11$			
x	Persistence length (nm)	(a) $\langle r^2 \rangle_x$ (nm) ²	(b) $\langle r^2 \rangle_x$ (nm) ²	x	Persistence length (nm)	(a) $\langle r^2 \rangle_x$ (nm) ²	(b) $\langle r^2 \rangle_x$ (nm) ²
22	30	2.62	2.43	22	30	2.55	2.34
25	35	3.37	3.07	24	35	3.28	2.95
27	40	4.22	3.78	27	40	4.10	3.56
30	45	5.11	4.50	29	45	4.90	4.25
32	50	6.02	5.20	31	50	5.74	4.86
33	55	6.94	5.86	33	55	6.61	5.47
36	60	7.85	6.47	35	60	7.50	6.03
40	70	9.88	7.79	39	70	9.34	7.15
47	90	14.09	10.03	45	90	13.18	8.94
53	110	18.40	11.56	51	110	17.13	10.02

Root-mean-square radius

The relatively small value of this parameter (109.5 nm) indicates a fairly flexible molecule: a very qualitative support for the value of the persistence length found. The result shows that there are about 20 Kuhn statistical elements/molecule which would mean a fairly flexible molecule; if there were only one-half that number, with a persistence length of about 80 nm, a rather-rigid molecule would seem plausible.

A somewhat more quantitative link is possible. The Kuhn statistical element length ($1/\lambda$) is the equivalent bond length which allows a molecule to be treated as a freely jointed chain. Then (from Tanford, 1961):

$$Rg^2 = m(1/\lambda)^2/6 \quad (10)$$

for a linear chain and m is the number of statistical elements in a molecule, if m is sufficiently large for

statistical analysis. Casassa (1965) has calculated that Rg^2 for a linearly freely jointed chain is just twice that for the equivalent circular molecule. Therefore:

$$Rg^2 (\text{circle}) = m(1/\lambda)^2/12 \quad (11)$$

By using $1/\lambda = 82$ nm and $m = 20$, Rg for RF II is calculated as 106.1 nm which is gratifyingly close to the experimental value of 109.5 nm. The persistence length found is, therefore, quite compatible with the experimental root-mean-square radius.

Second virial coefficient

Our results indicate a value of the second virial coefficient, B , of approximately zero. Harpst *et al.* (1968) and Dawson & Harpst (1971) found slightly positive values of B in the same buffer, for high-molecular-weight DNA and Dawson & Harpst (1971)

Fig. 3. Comparison of theoretical reciprocal particle-scattering factor, $P(\theta)^{-1}$, with the experimental one for $\epsilon = 0.141$

The solid lines with numbers are theoretical curves for the indicated persistence lengths, in nm. The points are experimental values obtained by pooling results from two separate experiments for the extrapolation to zero concentration and zero angle. The two sets of points were totally compatible. By visual examination a value of 41 ± 3.5 nm was estimated for the persistence length.

found bacteriophage λ circular DNA to have a marginally higher value than bacteriophage λ linear DNA. Other workers, however, have found a value of B of zero in similar solvents for linear DNA (Froelich *et al.*, 1963; Reichmann *et al.*, 1954; Cohen & Eisenberg, 1966) and there may be molecular-weight dependence for B , as is observed for other polymers such as polystyrene (Berry, 1966).

Jolly & Campbell (1972) previously obtained a slightly negative value of B for RF I DNA; if this is attributable, as suggested, to slight exposure of hydrophobic bases caused by some hydrogen-bond rupture in the supercoiled DNA, the disappearance of this negative value for relaxed circular DNA is as expected.

Fig. 4. Comparison of theoretical reciprocal scattering factor, $P(\theta)^{-1}$, with the experimental one for $\epsilon = 0.11$

The meaning of points, lines and numbers is as in Fig. 3. This value of ϵ does not give as good a fit to the experimental curve shape as Fig. 3.

Persistence length and function $P(\theta)$

The correctness of the experimental $P(\theta)^{-1}$ versus $\sin^2(\theta/2)$ curve is closely related to the correctness of extrapolation to 0° . As discussed, we believe this to have been accomplished with some precision. The other source of large error discussed by Schmid *et al.* (1971), anisotropy, is negligible in this system. The value of 41 ± 3.5 nm for the persistence length seems to be reasonably accurate for further reasons. First, the result has been obtained from the shape of the whole scattering curve from 30° to 150° , and when this value is used to calculate the root-mean-square radius of the molecule, the result matches very well with the value obtained experimentally from the initial slope; it seems more convincing, however, to use all the points in the scattering curve, rather than just the initial ones, to predict the persistence length. Objections to this (e.g. Hays *et al.*, 1969) have been on the grounds of the lack of precision of previous forecasts (e.g. Geiduschek & Holtzer, 1958). This,

however, was due to the extrapolation difficulty and the use of polydisperse systems; removal of these problems improves both methods, without changing their relative merits. Secondly, as it is a small molecule, the curves of $P(\theta)^{-1}$ are insensitive to changes in the excluded-volume factor (cf. Figs. 3, 4) rendering an exact knowledge of this parameter unimportant. Thirdly, the value is in good agreement with values from hydrodynamic techniques (Triebel *et al.*, 1971; Hearst *et al.*, 1968b; Gray *et al.*, 1967).

Conclusion

Although the range of DNA molecular weights found in naturally occurring forms is very wide and ranges from about 0.9×10^6 (Lee & Davidson, 1970) up to an unknown figure above 10^9 , the most readily available DNA molecules have been those from the bacteriophages and although small bacteriophage molecules are available and are more convenient as they are less susceptible to shear they are less readily prepared in the quantities required for physical experiments than are those from T7, T4 and λ bacteriophages. Conventional light-scattering instruments are limited in their application to these large DNA molecules because they cannot be employed at low enough angles to obtain accurate extrapolations. Possibly the development of the laser-scattering techniques will allow this problem to be overcome in the future and these molecules can be used for detailed studies of the solution structure of the giant worm-like DNA coils and their folding and unwinding. Until then, however, it seems to be worthwhile to characterize the solution structure of a DNA molecule which is small enough to permit accurate light-scattering extrapolations, homogeneous in size, and large enough to be treated by the well-documented theories surrounding the worm-like coil. The calculations and results presented in the present paper indicate that when a molecule within the right size range is investigated and appropriate alterations are made to accepted theories to allow for the fact that this molecule is circular, then light-scattering is indeed a valid technique for solution investigations on DNA molecules and may prove more suitable than the ultracentrifuge for studies on protein-nucleic acid interactions in some cases, since the weight-average molecular weight can be determined directly. We have also obtained evidence from light-scattering that the hydrodynamic results on the worm-like coil lead to the more correct persistence length.

The calculations involving the circularity of the molecule and the use of a circular molecule for experimental work seems to be valid and worthwhile since circular molecules are widely distributed in nature (Helinski & Clewell, 1971), and few small

DNA molecules are not circular. Since it is likely that small DNA molecules will be used in the expanding field of protein-nucleic acid interactions where the minimum disparity in size among the interacting components is preferable, this type of molecule together with its superhelical counterpart in form I appears to be very suitable for such a role.

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References

- Berry, G. C. (1966) *J. Chem. Phys.* **44**, 4550-4564
- Bloomfield, V. A. & Zimm, B. H. (1966) *J. Chem. Phys.* **44**, 315-323
- Casassa, E. F. (1965) *J. Polym. Sci. Part A* **3**, 605-614
- Cohen, G. & Eisenberg, H. (1966) *Biopolymers* **4**, 429-440
- Cohen, G. & Eisenberg, H. (1968) *Biopolymers* **6**, 1077-1100
- Daniels, H. E. (1952) *Proc. Roy. Soc. Edinburgh, Sect. A* **63**, 290-296
- Dawson, J. R. & Harpst, J. A. (1971) *Biopolymers* **10**, 2499-2508
- Eisenberg, H. (1969) *Biopolymers* **8**, 545-551
- Flory, P. J. (1969) *Statistical Mechanics of Chain Molecules*, p. 401, Interscience, New York
- Freifelder, D. (1970) *J. Mol. Biol.* **54**, 567-577
- Froelich, D., Strazielle, C., Bernardi, G. & Benoit, H. (1963) *Biophys. J.* **3**, 115-125
- Geiduschek, E. P. & Holtzer, A. (1958) *Advan. Biol. Med. Phys.* **6**, 431-551
- Gray, H. B., Jr. & Hearst, J. E. (1968) *J. Mol. Biol.* **35**, 111-129
- Gray, H. B., Jr., Bloomfield, V. A. & Hearst, J. E. (1967) *J. Chem. Phys.* **46**, 1493-1498
- Harpst, J. A., Krasna, A. I. & Zimm, B. H. (1968) *Biopolymers* **6**, 585-594
- Hays, J. B., Magar, M. E. & Zimm, B. H. (1969) *Biopolymers* **8**, 531-536
- Hearst, J. E. & Stockmayer, W. H. (1962) *J. Chem. Phys.* **37**, 1425-1433
- Hearst, J. E., Beals, E. & Harris, R. A. (1968a) *J. Chem. Phys.* **48**, 5371-5377
- Hearst, J. E., Schmid, C. W. & Rinehart, F. P. (1968b) *Macromolecules* **1**, 491-494
- Helinski, D. R. & Clewell, D. B. (1971) *Annu. Rev. Biochem.* **40**, 899-942
- Hermans, J. J. & Ullman, R. (1952) *Physica (Utrecht)* **18**, 951-971
- Jennings, B. R. & Plummer, H. (1970) *Biopolymers* **9**, 1361-1372

- Jolly, D. J. & Campbell, A. M. (1972) *Biochem. J.* **128**, 569–578
- Krasna, A. I. (1970) *Biopolymers* **9**, 1029–1038
- Kratky, O. & Porod, G. (1949) *Rec. Trav. Chim. Pays-Bas* **68**, 1106–1122
- Kuhn, W. (1936) *Kolloid-Z.* **76**, 258–271
- Kuhn, W. (1939) *Kolloid-Z.* **87**, 3–12
- Lee, C. & Davidson, N. (1970) *Biochim. Biophys. Acta* **204**, 285–295
- Peterlin, A. (1955) *J. Chem. Phys.*, **23**, 2464–2465
- Reichmann, M. E., Rice, S. A., Thomas, C. A. & Doty, P. (1954) *J. Amer. Chem. Soc.* **76**, 3047–3053
- Schmid, C. W. & Hearst, J. E. (1969) *J. Mol. Biol.* **44**, 143–160
- Schmid, C. W., Rinehart, F. P. & Hearst, J. E. (1971) *Biopolymers* **10**, 883–893
- Sharp, P. & Bloomfield, V. A. (1968a) *J. Chem. Phys.* **48**, 2149–2155
- Sharp, P. & Bloomfield, V. A. (1968b) *Biopolymers* **6**, 1201–1211
- Sinsheimer, R. L. (1959) *J. Mol. Biol.* **1**, 43–53
- Tanford, C. (1961) *Physical Chemistry of Macromolecules*, p. 167, John Wiley and Sons, New York
- Thomas, C. A., Jr. & MacHattie, L. A. (1967) *Annu. Rev. Biochem.* **36**, 485–518
- Triebel, H., Reinert, K. E. & Strasburger, J. (1971) *Biopolymers* **10**, 2619–2621
- Ullman, R. (1968) *J. Chem. Phys.* **49**, 5486–5497
- Weill, G., Hornick, C. & Toylov, S. (1968) *J. Chim. Phys. Physicochim. Biol.* **65**, 182–187
- Zimm, B. H. (1948) *J. Chem. Phys.* **16**, 1093–1116
- Zimm, B. H. & Stockmayer, W. H. (1949) *J. Chem. Phys.* **17**, 1301–1314