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ANALYSIS OF LOW ANGLE LIGHT SCATTERING RESULTS FROM T7 DNA

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Selected light scattering data, obtained in earlier studies on T7 DNA in 0.195 M Na^+, are analyzed by comparison with calculations from the theory of wormlike coils, both with and without excluded volume effects. The results confirm the conclusion from an earlier criticism, that linear extrapolations of data from the 10° to 20° angular range give incorrect values for the limiting molecular weight, M_T, and for the limiting root-mean-square radius, R_T. Further, it is shown that the excluded volume parameter, ϵ, must be used to provide a proper fit of calculated curves to experimental data. The revised analysis gives the following parameters for T7 DNA: $M_T = 25.5 \times 10^6$; $R_T = 587$ nm; $\epsilon = 0.08$; and the statistical segment length, $1/\lambda' = 120$ nm. These parameters agree well with other values in the literature. The method of analysis, therefore, provides reliable results from light scattering data on high-molecular-weight, native DNA.

1. Introduction

During the past quarter-century, the properties of DNA have been of special interest to physical chemists and biochemists, because of the unique behavior of DNA in solution and its biological function. The suggestion by Watson and Crick [1] of the double-helical structure stimulated development of a molecular explanation of many properties of DNA, including its biological function in replication and genetics. The structure imparts to DNA a rigidity in solution, which is reflected in many of its physical properties [2–4]. However, the structural rigidity of DNA raises some biological questions that have been, or are, difficult to answer: (1) How does a long, relatively stiff piece of DNA get "packaged" into an extremely small volume, such as a cell nucleus or virus? (2) How do gene expression and regulation occur in such a tightly-packed molecule? During the past few years, remarkable progress has been made in describing the subunit structure of DNA in chromosomes in terms of ν-bodies [5] or nucleosomes [6], which consist of the DNA and histones [7]. Elucidation of this subunit structure, which has been summarized recently by Weintraub, Worcel and Alberts [7], indicates how DNA may be packaged in cells and suggests possible mechanisms for gene expression. However, this subunit structure of chromosomes raises the question how, in a physical or thermodynamic sense, DNA with its limited flexibility can be wound into the tight coils, required in the subunits.

In view of these developments, interest in the stiffness of the DNA helix and in methods to measure it has been renewed. The rigidity of DNA in solution has been established in a number of investigations, which have been reviewed recently [8,9]. Many of these results have been analyzed in terms of a "stiffness parameter", often taken as the persistence length for the wormlike coil of Kratky and Porod [10]. Another factor which is important for large DNA molecules is the excluded volume parameter, ϵ [8,9,11,12]. Both parameters can be estimated from the dependence of the sedimentation coefficients and intrinsic viscosities on molecular weight, M [8,9,13,14]. Rayleigh light scattering, used in this investigation, seems to be ideal for studies on DNA, because it provides direct measurements of M and of the root-mean-square radius [15], R, which can be related directly to the persistence length [8,9,16].

Although Rayleigh light scattering has been widely used in the past 30 years to study properties of polymer solutions [17], its applicability to DNA solutions has oscillated widely. Shortly after discovery of the double-helical structure [1], light scattering was applied to determinations of M and R. In 1958 Geiduschek

and Holtzer published a penetrating review, in which serious questions about the results on DNA were raised because of heterogeneity of samples, long angular extrapolations, and other factors [18]. Attention then turned to homogeneous bacteriophage DNAs and to solving technical problems with clarification and with extension of measurements to angles below 30°. These efforts were successful in solving some of the technical problems [19,20] and in establishing the need for light scattering measurements at lower angles, so that values of M were not underestimated [18,19, 21–23]. In 1971, on the basis of developments in the theory of wormlike coils [3,4,13,24,25] and establishment of the molecular weight of T7 DNA by a variety of methods [25,26], Schmid, Rinehart and Pearst strongly criticized the results obtained up to then by light scattering [25]. In order to meet, or avoid, these criticisms, some workers turned their attention to light scattering studies on DNAs with molecular weights well below ten million [27–29]. In view of these efforts and the recent discoveries concerning chromosome structure, it seems appropriate to re-examine earlier light scattering results on DNAs with molecular weights over ten million.

The present paper provides a detailed re-evaluation of earlier light scattering data on T7 bacteriophage DNA [21,22], based on the criticisms of Schmid et al. [25] and the theory of Sharp and Bloomfield [24]. The results confirm the earlier criticisms. In addition, comparison of the data with theory [24,25] shows that the excluded volume parameter must be used to interpret scattering curves properly. This comparison leads to an improved method of analyzing light scattering data above 10° on native DNA, and provides estimates of the persistence length and excluded volume parameter.

2. Experimental

The experimental results on native T7 DNA, used for this investigation, were taken from earlier work [21,22,30,31]. Two complete sets of light scattering data, each with three reliable concentrations, were used to construct reciprocal-intensity plots [15,32], from which the molecular weight, M, root-mean-sqaure radius, R, and second virial coefficient, A_2, were obtained [15,21,22,30,31]. These parameters were determined by linear extrapolation of data from the 10° to 20° range. Only data from DNA solutions clarified by filtration were used, because centrifuged samples gave much less reproducible results [21].

All results were calculated with the refractive index increment, $dn/dc = 0.166$ ml/g [22,23], which agrees within experimental error with other values in the literature [27,28,33]. Measurements were made at the wavelength in vacuo, $\lambda_0 = 546$ nm, in BPES buffer (0.006 M Na_2HPO_4, 0.002 M NaH_2PO_4, 0.001 M Na_2·EDTA, and 0.179 M NaCl, pH 6.8) [21,22] with a refractive index, $n_0 = 1.329_6$. These parameters give for the optical constant, $\mathcal{K}$, the value of $3.59_4 \times 10^{-7}$ for vertically polarized light [22].

3. Theoretical

In order to compare results calculated from theory with experimental data, the following equation [15] was used:

$$\frac{\mathcal{K}c}{\mathcal{R}_\theta} = \frac{P^{-1}(\theta)}{M} = \frac{1}{M}\left[1 + \frac{16\pi^2}{3\lambda^2} R^2 \sin^2(\theta/2) + \ldots\right], \quad (1)$$

which holds in the limit as concentration, c, approaches zero. $P^{-1}(\theta)$ is the reciprocal of the scattering function for large particles [15], $\mathcal{R}_\theta$ is the Rayleigh ratio at angle, θ, and λ is the wavelength in the scattering medium, λ_0/n_0. The objective of theoretical calculations of $P^{-1}(\theta)$, as described below, is to provide correct, limiting ($\theta = 0$) values of M and R, designated as M_T and R_T, where T = theoretical. As pointed out by Geiduschek and Holtzer [18] and by Schmid et al. [25], linear extrapolations of experimental measurements are expected to give M_T and R_T only if $P^{-1}(\theta) < 1.3$. The available data on T7 DNA [21,22], taken at angles above 10°, do not satisfy this condition [25]. Consequently, linear extrapolations from reciprocal-intensity plots of the data [15,21,22] give apparent values of M and R, designated here as M_A and R_A.

Theoretical calculations of $P^{-1}(\theta)$ were made from available equations, both with and without the excluded volume parameter, ϵ [8,9,24]. For calculations without ϵ, eq. (B-3) of Sharp and Bloomfield [24], as corrected by Schmid et al. [25], was used. In this case the value of R_T was calculated from eq. (1) of Hays, Magar and Zimm [16]. The persistence length, a [10, 16], is one-half the Kuhn [8,34] statistical segment length, $1/\lambda'$.

Calculations of $P^{-1}(\theta)$ with excluded volume were made from eq. (11) of Sharp and Bloomfield [24]. The most troublesome aspect of these calculations was evaluating the incomplete gamma function, $\gamma(\alpha,x)$, where α and x are defined in eq. (12) of Sharp and Bloomfield [24]. After appropriate ranges of the variables were determined, values of $\gamma(\alpha,x)$ were calculated from equations given in Abramowitz and Stegun [35]. The values were checked for accuracy against tabulated results [35,36]. Values of R_T when $\epsilon > 0$ were calculated as the square root of the following expression,

$$\langle R^2 \rangle = \frac{b^2 \lambda^{\epsilon-1} \sigma^{\epsilon+1}}{(\epsilon+2)(\epsilon+3)} \left[1 - \frac{\epsilon+3}{2\lambda\sigma(\epsilon+1)}\right], \qquad (2)$$

which will give R in cm and includes a corrected sign in the denominator [9,24,37]. Symbols are used as given by Sharp and Bloomfield [24]. Substitution of appropriate parameters [16,24] into eq. (2) for the case $\epsilon = 0$ gives the first two terms of eq. (1) of Hays et al. [16]. Values of ϵ and associated parameters, intermediate to those listed in table 1 of Sharp and Bloomfield [24], were obtained by graphical interpolation of their results.

In addition to the parameters mentioned above, two others were needed for the calculations. The parameter, b, in eq. (2) was the hydrodynamic diameter of native DNA and was taken as 2.7 nm [24]. The mass per unit length, m/ℓ, needed to calculate the chain length, L, from molecular weight can vary considerably for NaDNA in the B configuration [38]. Since similar calculations were needed for analyzing results on T2 bacteriophage DNA [31], the value, $m/\ell = 198_8$ daltons/nm, was taken as the average for both T7 and for glucosylated T2 DNA [38]. Calculations with the more widely-used value of 1950 daltons/nm [25] gave an insignificant change in the results, as expected [39]. All calculations involving lengths were done in cm units.

Theoretical scattering curves ($\mathcal{K}c/\mathcal{R}_\theta$) were calculated from eq. (1) and from values of $P^{-1}(\theta)$, determined with appropriate theoretical equations at intervals from one to five degrees for scattering angles between zero and seventy degrees. Approximate values of M_T, $1/\lambda'$, and ϵ were first assumed, and then adjusted after comparing the calculated values of $\mathcal{K}c/\mathcal{R}_\theta$ with experimental results. This comparison was facilitated by a Fortran IV computer program which provided linear, least-squares extrapolations of the calculated curves to give M_A and R_A from the same, limiting angular range (10–20°) used for linear extrapolations of experimental data [21,22]. In addition, computer-drawn graphs of $\mathcal{K}c/\mathcal{R}_\theta$ versus $\sin^2(\theta/2)$ were made from the calculated results for comparison by overlay with experimental data. These calculations and plotting were done on a Digital Equipment PDP 11/45 computer. Finally, to provide an objective measure of the fit of calculated curves to the experimental one, the sum of the squares of the deviations, Σd^2, has been determined, where d is the difference between calculated and experimental values of $\mathcal{K}c/\mathcal{R}_\theta$ at a given angle. The deviations were calculated by assuming equal weighting for points at all angles. Calculations of Σd^2 were made on a Texas Instrument TI-59 programmable calculator.

4. Results

As noted in the Experimental section, the light scattering data used in this paper were selected from earlier work [21,22,30,31] and corrected for the proper value of dn/dc. Extrapolations of reciprocal-intensity plots from the linear, and lowest accessible, 10–20° range gave values for T7 DNA of $M = (29.0 \pm 0.3) \times 10^6$ and $R = 709 \pm 2$ nm. The indicated uncertainty is the average deviation for the two sets of data. For the reason noted in the preceding section, these values of M and R are taken as apparent experimental ones, M_A and R_A, and are to be compared with calculated values derived from the same angular range and tabulated in tables 1 and 2.

Since the theoretical calculations are made for macromolecules assumed to have essentially no intermolecular interactions, it would be preferable to compare the calculated curves directly with the scattering curve at $c = 0$ from a reciprocal-intensity plot [21,32]. The theoretical curves for T7 DNA under conditions of these experiments should be accurate up to a 55° angle [37]. However, concentration extrapolations of experimental data become less reliable at angles above about 30°, because the measured light levels begin to approach the detection limit of the instrument, due to the asymmetry of scattering and the low DNA concentrations used. In spite of these problems with concentration extrapolations, the experimental angular envelopes for individual concentrations are all nearly par-

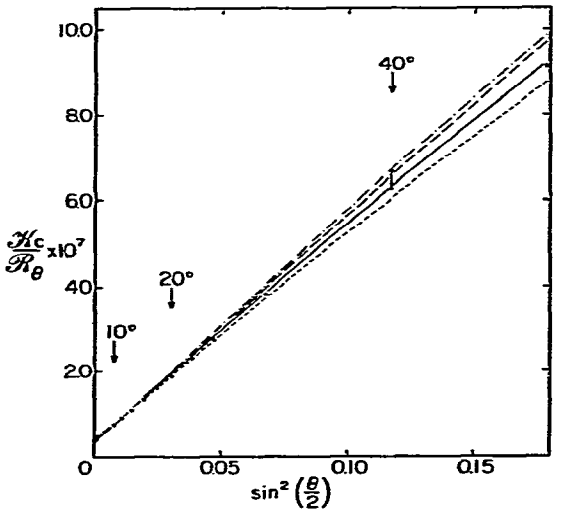

Fig. 1. Plot of $\mathcal{K}c/\mathcal{R}_\theta$ versus $\sin^2(\theta/2)$ at $c=0$ over the angular range of 0° to 50° for T7 DNA. Experimental points, derived as described in the text, are indicated by the filled circles (●) or bar (I). The diameter of the circles or length of the bar encompasses the difference between the two sets of data used at the indicated angles. The curves were calculated to give the best fits to 10–20° data at $c = 0$ from reciprocal-intensity plots with the following parameters: (——), $\epsilon = 0.08$, $M_T = 25.5 \times 10^6$, $1/\lambda' = 120$ nm; (– –), $\epsilon = 0.06$, $M_T = 25.0 \times 10^6$, $1/\lambda'$ [130 nm; (-----), $\epsilon = 0.10$, $M_T = 26.5 \times 10^6$, $1/\lambda' = 110$ nm; and (–·–·), $\epsilon = 0$, $M_T = 23.0 \times 10^6$, $1/\lambda' = 150$ nm.

allel to each other, and to the estimated curves at $c=0$, at least to 70° [31]. Consequently, it was decided to use experimental scattering envelopes at finite concentrations, translated to $c=0$ with appropriate second virial coefficients ($A_2 = 4.1_8 \times 10^{-4}$ mol cm^3/g^2 and $3.8_6 \times 10^{-4}$ mol cm^3/g^2 for the two sets of data) derived from the low-angle reciprocal-intensity plots [21, 22,30,31]. The experimental points used in fig. 1 are from measurements on DNA solutions at approximately 30 μg/ml from the two different sets of data, translated as described to $c=0$. The envelopes were selected to approximate the differences between all acceptable scattering curves obtained in the measurements. The experimental points selected in this manner have a slightly smaller slope (R) in the linear 10–20° region, than does the average of the limiting ($c=0$) extrapolations from the reciprocal-intensity plots for the two sets of data. The estimated, experimental scattering curve at $c=0$ is plotted in fig. 1.

Results from several scattering curves, calculated with $\epsilon=0$, are summarized in table 1. The table illustrates the best available agreement between calculated values of M_A and R_A and experimental ones ($M = 29 \times 10^6$, $R = 709$ nm) derived from the 10–20° extrapolation of reciprocal-intensity plots. The value of Σd^2, also shown in table 1, was obtained at 2° intervals for the 10–20° angular range as described in the Theoretical section 3. In this range, the experimental points used to calculate Σd^2 were averages taken from $c=0$ extrapolations of the reciprocal-intensity plots [21,2,30,31]. A minimum in Σd^2, indicating the best agreement between theoretical and experimental curves, is obtained with assumed values of $M_T = 23.0 \times 10^6$ and $1/\lambda' = 150$ nm. Table 1 illustrates the good quality and uniqueness of the agreement between the curves by inclusion of results from higher and lower values of both M_T and $1/\lambda'$. Closer agreement could be obtained between calculated and experimental results (table 1), but this is unnecessary because of the experimental uncertainty. The calculated curve giving the best fit in the 10–20° range with $\epsilon=0$ is the upper one shown in fig. 1.

In the high-angle (25–50°) region, it is difficult to fit curves calculated with $\epsilon=0$ to the experimental points. To make this comparison, values of Σd^2 (table 1) were calculated for 6 angles from 25° through 50°. The experimental points used in this range were averages of the experimental scattering envelopes at finite concentrations, translated to $c=0$ with the second virial coefficient, as described in an earlier paragraph. The values of Σd^2 (table 1) show that the theoretical curves can fit experimental points in the 25–50° range only by sacrificing agreement in the 10–20° range. Clearly, when the agreement is best in the 10–20° region, the calculated and experimental curves do not fit well at high angles. This case is illustrated by comparing the upper curve to the experimental points in fig. 1.

Table 2 summarizes the results from calculations with non-zero values of ϵ, in addition to those for M_T and $1/\lambda'$. The table shows that for each value of ϵ, the other parameters can be adjusted to fit calculated values of M_A and R_A from the 10–20° range to the experimentally-derived numbers ($M = 29 \times 10^6$, $R = 709$ nm). This agreement between calculated and experimental curves in the 10–20° range is reflected in the minima obtained for Σd^2 (table 2). Comparison of the results in table 1 with those in table 2 clearly shows

Table 1
Results [a] from calculated scattering curves for T7 DNA without excluded volume

M_T × 10^{-6}	$1/\lambda'$ (nm)	R_T (nm)	M_A [b] × 10^{-6}	R_A [b] (nm)	(Σd^2) [c] × 10^{14}	
					(10–20°)	(25–50°)
22.0	140	503	26.8	654	0.0198	0.0577
	150	521	27.2	684	0.0020	0.7216
	160	537	27.5	712	0.0423	2.3957
23.0	140	515	28.3	674	0.0289	0.0569
	150	533	28.7 [d]	704 [d]	0.0003	0.6978
	160	550	29.0	733	0.0303	2.3514
24.0	140	526	29.8	694	0.0395	0.0568
	150	544	30.2	724	0.0012	0.6767
	160	562	30.6	754	0.0217	2.3124

a) Symbols are used as defined in the text.
b) Apparent values of M and R are determined from the calculated curves by linear extrapolation from the 10° through 20° angular range.
c) Σd^2 is the sum of the squares of the deviations in $\mathcal{R}_c/\mathcal{R}_\theta$ between the theoretical and experimental curves for the angular ranges indicated. Further details are given in the text.
d) These values most closely coincide with experimental results.

that including ϵ in the calculations changes the values of M_T and $1/\lambda'$ needed to fit the experimental results. Although the results for the 10–20° angular range from table 2 provide information needed to select appropriate values of M_T and $1/\lambda'$ for any given ϵ, they provide no direct means for selecting a single value of ϵ.

The lower three curves in fig. 1 are those calculated with the three different values of ϵ in table 2, each of which provides a satisfactory fit to the experimental data in the 10–20° range. The most interesting result which emerges from a comparison of the calculated curves with experimental points between 10° and 50° is that the calculations with $\epsilon = 0.08$ provide the best

Table 2
Results [a] from calculated scattering curves for T7 DNA with excluded volume

M_T × 10^{-6}	ϵ	$1/\lambda'$ (nm)	R_T (nm)	M_A [b] × 10^{-6}	R_A [b] (nm)	(Σd^2) [c] × 10^{14}	
						(10–20°)	(25–50°)
25.0	0.06	110	537	28.8	658	0.1171	1.3051
		120	559	29.0	688	0.0234	0.1273
		130	580	29.1 [d]	716 [d]	0.0012	0.4256
25.5	0.08	110	564	28.7_9	676	0.0445	0.7040
		120	587	28.8_0 [d]	704 [d]	0.0003	0.0474
		130	609	28.7_7	729	0.0306	0.8158
26.0	0.08	110	570	29.4	684	0.0493	0.7137
		120	593	29.4	711	0.0008	0.0469
		130	615	29.3	736	0.0270	0.8053
26.5	0.10	100	575	29.1	671	0.0816	1.8454
		110	600	29.0 [d]	698 [d]	0.0071	0.3101
		120	623	28.9	722	0.0132	0.1484

a, b, c, d) Footnotes are the same as for table 1.

fit. This agreement, especially at the higher angles (25–50°), is indicated by a minimum in the value of Σd^2 (table 2), which parallels the minimum in the 10–20° range. Clearly the curves (fig. 1) with $\epsilon = 0$ and 0.06 have too little curvature above 20°, and that with $\epsilon = 0.1$ has too much curvature at the higher angles. These observations are substantiated by the relatively large values of Σd^2 found for curves calculated with these values of ϵ in the 25–50° range. Comparison of the calculated and experimental curves at the higher angles, after first obtaining a good fit in the 10–20° range, shows that $\epsilon = 0.08$ provides the best fit of the calculated curves to the experimental points (fig. 1, table 2) over the entire, 10° to 50° range.

5. Discussion

One difficulty associated with this work was the evaluation of experimental results and their selection for comparison with theoretical calculations. In their earlier analysis, Schmid et al. [25] used the results of Harpst et al. [21] from T7 DNA, clarified both by centrifugation and by filtration. As shown in their fig. 2, Schmid et al. [25] used all three sets of data on T7 DNA from table 1 of Harpst et al. [21], and fitted their calculated curve to what appeared to be the intermediate one of the three (see fig. 2, ref. [25]). This curve was from a centrifuged sample which, unfortunately, also had an unusually high M_A and R_A (cf. table 1 of Harpst et al. [21]). Later work in this laboratory [22,31,40] and by other investigators [23,27–29,41,42] confirmed that filtration was the preferred method of clarification. Therefore, only data from filtered samples of T7 DNA [21,22,30,31] were selected for comparison with calculated curves.

The method for obtaining an experimental scattering curve at $c = 0$ over the 10–70° angular range was described in Results. This method provides the experimental curve (fig. 1) with a slope in the 10–20° range which is 1.6% less than the average of the limiting slopes from reciprocal-intensity plots. This difference is well within experimental uncertainty; an illustration of the small difference can be seen in fig. 2 of Harpst et al. [21]. At present the validity of experimental points above 20° (fig. 1) is an assumption, borne out, in part, by the good agreement between different sets of data, and by the fit to calculated curves as discussed below. Apparent differences in second virial coefficients, derived from low-angle and from high-angle data [21], suggest that extrapolations to $c = 0$ in reciprocal-intensity plots might provide an envelope quite different from experimental curves at finite concentrations, as used here. However, as observed by Harpst et al. [21], the virial coefficients from high-angle data are variable and reflect the uncertainty in high-angle data, noted in Results.

Schmid et al. pointed out two other potential problems with light scattering data on DNA [25]. One was the effect of anisotropy on R_A. For the data in this paper it has been assumed that the anisotropy effect is negligible. This assumption appears reasonable for high-molecular-weight T7 DNA in view of the recent finding by Godfrey and Eisenberg [28] that the effect of anisotropy decreases and probably approaches zero for DNAs with $M > 10^6$. The second problem was the effect of heterogeneity of the DNA on curvature of the scattering envelope [18]. In this study the problem is avoided by using whole, homogeneous, T7 DNA [21,22,38].

The closest fit of curves calculated with $\epsilon = 0$ to the 10–20° experimental data (table 1, fig. 1) gives $M_T = 23 \times 10^6$, $R_T = 533$ nm, and $1/\lambda' = 150$ nm. As found by Schmid et al. [25], M_T from the calculated curve is about 20% lower than the value of 29×10^6 from reciprocal-intensity plots. However, M_T from these calculations is 8% lower than the estimated value of 24.9×10^6 obtained previously [25], because the fit in the present study is to data on filtered DNA alone. In addition the calculated M_T is 8% below and is just outside the expected uncertainty in the accepted value of $(25.2 \pm 0.6) \times 10^6$ for T7 DNA [8,25,26,38]. The calculated $R_T = 533$ nm agrees closely with the theoretical value, 522 nm, obtained by Schmid et al. [25] and is significantly lower than the experimental value, $R_A = 709 \pm 2$ nm, obtained by linear extrapolation of 10–20° data in reciprocal-intensity plots. This comparison of radii more clearly illustrates the upward curvature at angles below 10° [24,25] than is visible in fig. 1, due to the choice of scales. The value for $1/\lambda' = 150$ nm (persistence length, $a = 75$ nm) is considerably higher than that (130 nm) obtained by Schmid et al. [25], probably because they chose to fit calculations to the data on centrifuged samples, as discussed above. The statistical segment length of 150 nm found here with $\epsilon = 0$ is significantly higher than

the value of 120 nm determined by Hays et al. [16] from hydrodynamic data, and is greater than the presently-accepted range of 120–140 nm [8,9,27,28]. These comparisons, particularly of M_T and $1/\lambda'$, with other results in the literature and the failure of the curve calculated with $\epsilon = 0$ to fit the experimental data above 20° (fig. 1, table 1) suggest that the results without excluded volume are not accurate.

In the Results section a comparison of calculations, incorporating the excluded volume parameter, with experimental data led to determination of the following parameters, which provide the best fit to both the experimental values of M_A and R_A and to the experimental curvature between 10° and 55°: $M_T = 25.5 \times 10^6$, $\epsilon = 0.08$ and $1/\lambda' = 120$ nm. Use of the excluded volume parameter significantly reduced the value of $1/\lambda'$, contrary to that expected from some considerations [16], but consistent with others [8,9,13]. The lower value $1/\lambda' = 120$ nm (or $a = 60$ nm), is in close agreement with the results of other workers [8,9,16, 27,28], discussed in the preceding paragraph. Such close correspondence suggests that the persistence length for native DNA is constant for molecular weights ranging from a few hundred thousand [8,27,28] to at least 25×10^6, if the excluded volume is taken into account. A more recent determination of the persistence length by light scattering ($a = 50$ nm) [43] is even lower than the present results with excluded volume.

The comparison of calculated and experimental curves provides a method for evaluating ϵ. Although the parameter cannot be determined with great accuracy, the result, $\epsilon = 0.08 \pm 0.01$, from fig. 1 and table 2 appears reasonable. The uncertainty is relatively large, but it is clear both from fig. 1 and from the values of Σd^2 in table 2 that the previously-used value of 0.11 [24] is too great. The value, 0.08, agrees remarkably well with the result, $\epsilon = 0.072 \pm 0.036$, obtained by Hearst et al. from hydrodynamic studies [13].

Another result of the calculations with $\epsilon = 0.08$ is that the value of $M_T = 25.5 \times 10^6$, obtained from the best fit to experimental data, agrees well with the accepted value of 25.2×10^6 for T7 DNA [8,25,26,38]. The difference between the value of $M_T = 25.5 \times 10^6$, derived with excluded volume (table 2) and the result $M_T = 23 \times 10^6$ without excluded volume (table 1) appears to be significant. The implication is that the excluded volume parameter must be taken into account to fit properly the experimental data and to give reliable values of M_T and $1/\lambda'$. Use of the value of $M_T = 25.5 \times 10^6$ shows that early-time measurements on T7 DNA denatured by acid give a molecular weight nearer to one-half the native value than was indicated in a previous study [22].

Comparison of theoretical calculations with experimental light scattering results, as described in this paper, leads to the following conclusions:

(1) In agreement with the conclusions from earlier critiques [24,25] this work confirms that the previous use of linear extrapolations of light scattering data from the 10–25° region [21–23] was incorrect for T7 DNA.

(2) The procedures used here provide a method for obtaining reliable, limiting values of M and R, even though the light scattering data are not extended to low enough angles for making valid, linear extrapolations.

(3) Incorporation of the excluded volume parameter, ϵ, as described by Sharp and Bloomfield [24], is essential for obtaining the correct molecular weight, M_T, and for properly fitting calculated curves to experimental data, especially at angles from 20° to 50°.

(4) The curve-fitting procedures give a value of $\epsilon = 0.08$ for T7 DNA, and indicate how the parameter may be evaluated in future studies.

(5) The Kuhn statistical segment length, or persistence length, can be evaluated by the method described, and appears to be reliable when the proper value of ϵ is used.

(6) The analysis provided here for light scattering data on high-molecular-weight T7 DNA brings the results into excellent agreement with those obtained by hydrodynamic and other methods.

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