LIGHT-SCATTERING STUDIES OF SODIUM DESOXYRIBONUCLEATE

by

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INTRODUCTION

Recent studies of the molecular weight, size and shape of sodium desoxyribonucleate (DNA) have led to a rather large range of molecular weight values ($0.50–24.0 \times 10^6$) and three different deductions concerning the molecular configuration. For example SMITH AND SHEFFER[1] concluded on the basis of light-scattering studies that the molecular weight of three of their samples varied from $3.7–24 \times 10^6$. They also concluded that the configuration of these three samples varied from that of a partially coiled to a randomly coiled configuration. OSTER[2] also examined three samples and concluded that they had a range of 0.5 to 3.5 million and the molecule was a rod. DOTY[3] has studied a sample having a molecular weight of 8 million and a configuration intermediate between that of a rod and a coil. On the other hand BUTLER AND JAMES[4] have concluded on the basis of diffusion and ultracentrifuge studies that DNA forms, even in dilute solutions, a three-dimensional gel network. Consideration of the above and of additional studies of molecular parameters[5,6,7,8,9,10] strongly suggests that there is a large sample to sample and technique to technique variation. It is the purpose of this and subsequent reports to describe studies by different techniques (light-scattering, electron microscopy, and viscometry) of a highly polymerized sample of DNA as a first step in resolving the above views.

In studying the effect of enzyme action on the light-scattering properties of dilute solutions of DNA, it was observed that the sample contained an enzyme resistant fraction. This fraction was separated from the products of the enzyme action and studied by light-scattering and infrared spectroscopy.

In addition, the effect of the ionic strength of the solution on the light-scattering and flow birefringence properties of the solution of undegraded DNA was investigated.

This first report*** deals primarily with the light-scattering experiments. The following reports will present the results of the electron microscopy and viscometry studies.

EXPERIMENTAL METHOD

The sample of sodium desoxyribonucleate was prepared by the method of GULLAND *et al.*[11]. It had a nitrogen content of 15.9%, a phosphorus content of 9.1%, and a protein content of 0.6%. When dissolved in water the fibers gave rise to clear viscous solutions having a sedimentation constant

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of 9.78 ∓ 0.22 (0.1 % solution in 3 *N* KCl). This solution may be kept for several weeks without change at 0° C.

The light-scattering apparatus was built by the American Instrument Co. It was calibrated by measuring the turbidity of 3 solutions at wave-length 436 mμ and checking one against the other. The three solutions are as follows:

a. A high molecular weight polystyrene sample in toluene. The turbidity of the sample was measured on the absolute camera in Dr DEBYE's laboratory, and it was obtained through the kindness of Dr. R. F. STEINER.

b. A recrystallized sample of Armour's serum albumin having a molecular weight of 70,000.

c. Four dilutions of a fresh DNA solution whose turbidities had been determined on a calibrated SPEISER-BRICE apparatus[12].

All three solutions gave good agreement for the instrument constant. The molecular weight of the serum albumin was 72,000 ± 3,000 when determined by this instrument constant.

ZIMM's double extrapolation method[13] was found to be applicable to the scattering of light for dilute solutions of DNA.

The infrared spectra were obtained on films of native and enzymically degraded sodium desoxyribonucleate solutions which were prepared by evaporation of 2 ml (5 mg/ml) of solution on 25 × 5 mm uncoated silver chloride disks. The spectrum of these strong tough films of DNA was obtained on a double beam, Model 21, PERKIN-ELMER infrared spectrometer[14] with the resolution set at 4 and the gain at 6.3 units. Sodium chloride optics were employed and the wave-length scale was calibrated by the use of the carbon dioxide and water vapour bands. The calibration was accurate to ± 0.02 micron.

The birefringence measurements were made on an apparatus of the type described by EDSALL[15]. The gap width was 0.10 mm. The rotary diffusion coefficient was obtained (assuming the elongated ellipsoid of revolution model) from the following Perrin relationship:

$$\theta = \frac{3\,KT}{16\pi\eta a^3}\left[-1 + 2\ln\frac{2a}{b}\right]$$

Where K is the Boltzmann's constant, η is the viscosity of the solution in poises and a and b are major and minor axes of the ellipsoid. In computing θ, a/b was set at 300, a value close to the one obtained from our viscosity measurements on the GULLAND sample.

The ratio of G/θ ($= \alpha$), where G is the shear rate and θ the rotary diffusion constant, as a function of extinction angle, was obtained from tables computed on the basis of the theory of PETERLIN AND STUART[16].

MOLECULAR WEIGHT AND LENGTH

Ten ml of 5 mg/ml solution of DNA in 3× distilled water was placed in a 7 mm diameter dialysis bag. The sample was dialyzed against 3 liters of 3× distilled water, with frequent changes of water, for a 24-hour period. The small amount of denatured DNA caused by dialysis was removed by centrifugation. At the end of this time the light scattering envelope of dilute solutions was determined. In five separate determinations the parameter[17] $P(\theta) = I_\theta/I_o$ defining the envelope, (where I_θ is the reduced intensity at any angle and I_o is the scattering at 0°) at angles between 40° and 90° was intermediate between the theoretical curve for the rod and for the coil.

The observed measurements are shown for a typical case in Fig. 1. Where the factor $P(\theta)$ is plotted versus the sine of the angle of observation, the length chosen for the theoretical curve was obtained from the limiting-slope of the Zimm plot described below. Examination of the scattering envelope of a great many solutions of the DNA of varying ionic strength showed that in all cases the configuration is intermediate between that of a rod and a coil. Fig. 2 is a typical scattering envelope for a solution of ionic strength of of 1.0.

ZIMM's method of plotting the data employs a double extrapolation, and, as shown in Fig. 3, one plots

$$\frac{HC}{\tau} \text{ versus } \sin^2\theta/2 + kc$$

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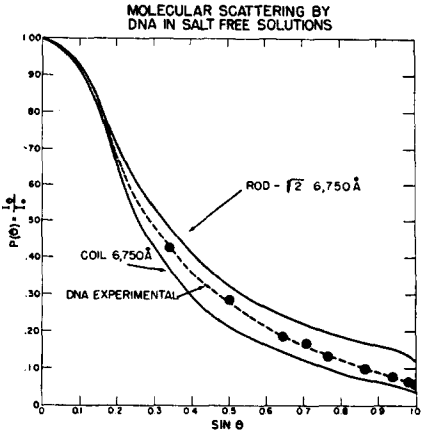

Fig. 1. Theoretical and experimental correction terms for dialyzed DNA solution as a function of the sine of the angle of observation. Length values for the theoretical curves are obtained from corresponding ZIMM plots similar to Fig. 3.

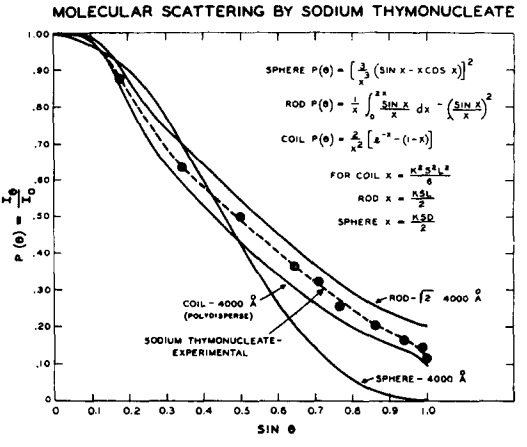

Fig. 2. Theoretical curves for rod, coil and sphere. Solid dots are experimental points for DNA at ionic strength of 1.0.

where H is the refraction constant, C is the concentration (in grams/ml), τ is the turbidity, θ is the angle of observation and k is an arbitrary constant. ZIMM uses a constant K in the left hand member which is $\frac{3}{16\pi} H$ and shows that the correction term $P(\theta)$ for large particles may be expressed in terms of a power series:

$$1.\quad P(\theta) = 1 - \frac{c}{3} L^2 \sin^2 \theta/2 + \frac{c^2}{12} L^4 \sin^4 \theta/2 +$$

where $c = 8\pi^2/3\lambda^2$, λ the wave-length of light and L = length of scattering particle. The correction term, the scattering and the molecular weight are related by the equation[18]

$$2.\quad \frac{HC}{\tau} = \frac{1}{M} P(\theta) + 2BC$$

where M is the molecular weight and B is the first coefficient of VAN 'T HOFF's equation.

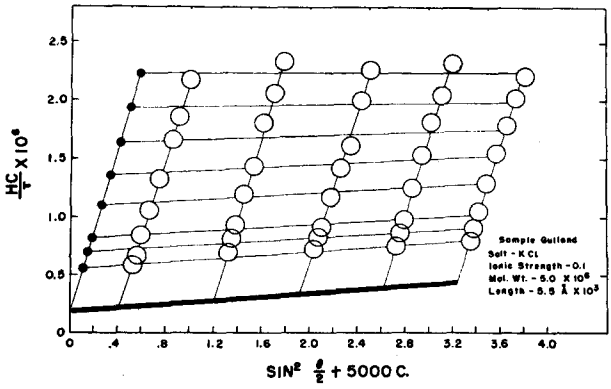

Fig. 3. Typical ZIMM plot for dilute DNA solution. Angles of observation are from 40°–100°.

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It is thus possible to obtain from a single plot, using double extrapolation, two different limiting slopes which lead to values of L and M. One double extrapolation plot typical of some 20 experiments is shown in Fig. 3. In this experiment at an ionic strength of 0.1 and DNA concentration of $1\text{–}7 \cdot 10^{-4}$ g/ml a length based on a coil model of 5,500 A and weight of 5 million was obtained. The length is obtained by dividing the square root of the slope divided by the intercept of the $\frac{HC}{\tau}$ versus $\sin^2 \theta/2$ multiplying by $1.105 \cdot 10^3$ as follows:

$$3.\ L = \left(\sqrt{\frac{\text{slope}}{\text{intercept}}}\right)(\lambda)\left(\sqrt{\frac{9}{8\pi^2}}\right) = \left[\left(\sqrt{\frac{\text{slope}}{\text{intercept}}}\right)(1.105 \cdot 10^3)\right]$$

For a rod model the relationship is the same except that there is an additional factor of $\sqrt{2}$.

In order to check the effect of the ionic strength on the computed value of the molecular weight and the molecular length, the experiment of Fig. 3 was repeated for a number of solutions of varying ionic strengths; the results of these measurements are shown in Table I.

In the experiments summarized in Table I the ionic strength varied from 0–2.5 and the DNA concentration varied from $0.4\text{–}7.0 \cdot 10^{-4}$ g/ml. There appears to be a slight downward trend of the molecular weight values with increasing ionic strength. The average for this sample is $4.51 \pm 0.53 \cdot 10^6$. The molecular length was definitely dependent upon ionic strength falling from 6.8 to $4.2 \cdot 10^3$ A, a length decrease of 39 %. The average value of length in salt solutions was $4.58 \pm 0.58 \cdot 10^3$ A on a coil model and $6.50 \cdot 10^3$ A on a rod model.

TABLE I

MOLECULAR LENGTH AND WEIGHT OF SODIUM THYMONUCLEATE

Exp. No.	*Solvent*	*Ionic strength* $\frac{1}{2}$	*Conc. range* $g/ml \times 10^4$	I_{45}/I_{135} $C \rightarrow O$	*Molecular Weight* $\times 10^{-6}$	*Molecular Length* $\times 10^{-3}$ *A*
1	H_2O	0	0.40–1.5	2.8	4.55	6.75
2	H_2O	0	0.74–5.3	3.0	5.00	6.99
3	$1.20 \cdot 10^{-4}$ *M* KCl	$1.10 \cdot 10^{-2}$	0.40–1.5	3.6	4.16	4.98
4	$1.20 \cdot 10^{-3}$ *M* KCl	$3.47 \cdot 10^{-2}$	0.40–1.3	3.5	3.86	4.86
5	$1.62 \cdot 10^{-2}$ *M* KCl	$1.27 \cdot 10^{-1}$	0.79–2.9	3.7	5.80	5.15
6	$1.00 \cdot 10^{-1}$ *M* KCl	0.346	1.00–7.0	3.5	5.00	5.56
7	$5.00 \cdot 10^{-1}$ *M* KCl	0.706	2.50–5.0	3.1	4.01	3.62
8	1.0 *M* KCl	1.00	2.50–5.0	3.6	4.55	4.08
9	1.0 *M* $MgCl_2$	1.73	1.00–3.5	3.5	4.81	4.20
10	2.15 *M* $MgCl_2$	2.54	1.00–5.5	3.6	3.37	4.15
					4.51 ± 0.53	4.58 ± 0.58

It is noted in Table I that the intrinsic dissymmetry is slightly smaller for the dialyzed samples and ranges from 3.1 to 3.7. This range of values is greater than the maximum value for an infinitely long rod —2.5; it is also greater than the maximum value for an infinitely long negatively anisotropic rod —2.8.[19] These values support the concept that the nucleic acid molecule is a flexible rod and not that of a stiff rod like the tobacco mosaic virus[17].

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In Fig. 4 there is plotted the coil model length values of Table I versus the square root of the ionic strength. The first two points, obtained on carefully dialyzed solutions, are approximately 39% greater than the points obtained at the higher ionic strengths. These values tend to suggest that there is a diminution in molecular length as the ionic atmosphere surrounding the molecule is increased. In view of this effect it was of interest to see if there was a length change upon addition of salt in the flow birefringence experiment[15]. The extinction angle of the same dialyzed sample of DNA was measured as a function of velocity gradient. Upon the addition of a small amount of KCl ($3 \cdot 10^{-2}$ M) there was a marked and reproducible increase in extinction angle. This effect is shown in Fig. 5. We note a decrease in length of about 36%—a value in good agreement with the length decrease (39%) detected by the light scattering technique.

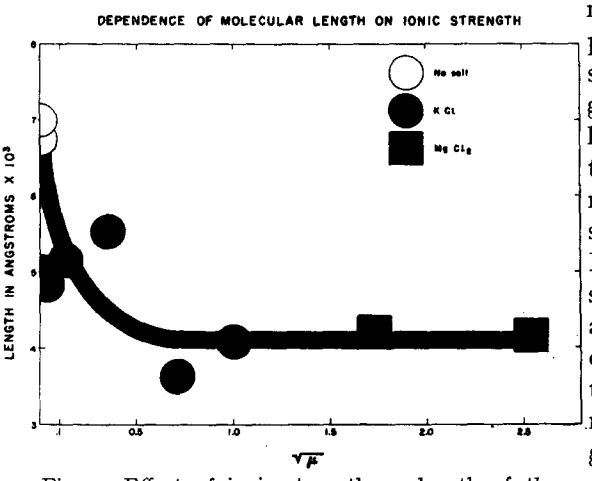

Fig. 4. Effect of ionic strength on length of the DNA molecule. The open circles are on separately weighed and dialyzed solutions. Lengths are computed on a coil model.

MOLECULAR SHAPE

It is of interest to compare the following molecular lengths obtained by the two techniques, assuming 3 different models, light-scattering, rod—$9.6 \cdot 10^3$, coil $6.8 \cdot 10^3$ flow birefringence, prolate ellipsoid —$4.7 \cdot 10^3$ in salt free solution. The difference in values obtained by the two methods is undoubtedly, in part, due to the assumption of different models used. The equivalent percentage decreases in molecular length obtained by the two very different methods strongly support the hypothesis that the DNA molecule undergoes shortening when surrounded by the electrical charges of neighbouring ions. The theory of dimensional change of charged long chain molecules proposed by HERMANS AND OVERBEEK[20] leads to curves of length versus ionic strength which are similar in shape and magnitude to the curve of Fig. 4. The data of Figs. 4 and 5 and the similarity between experimental observations and above-mentioned theory strongly support the concept that this rod-like charged molecule is flexible enough to undergo shortening when surrounded by the charged ions.

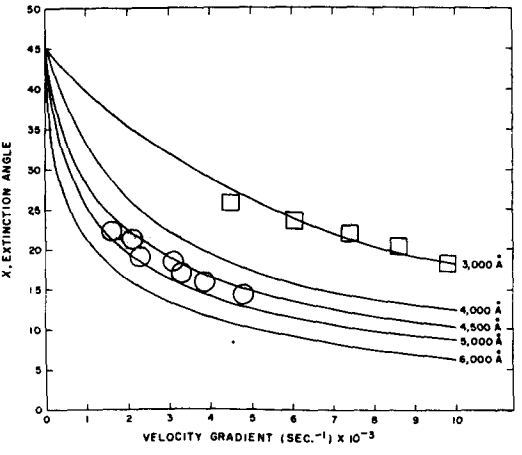

Fig. 5. Dependence of extinction angle on shear rate. Addition of $3 \cdot 10^{-2}$ M salt results in higher extinction angle. The DNA concentration here is $5 \cdot 10^{-4}$ g/ml.

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GEL-LIKE BEHAVIOUR

The data thus far supports the picture deduced by DOTY *et al.*[3,34] that the configuration of the DNA molecule is intermediate between that of a rod and a coil. In view of the hypothesis put forth by BUTLER AND JAMES[4] that DNA forms in dilute solutions a three-dimensional network it was of interest to examine the light-scattering from this point of view. In the following we have analyzed the light-scattering data in terms of the FLORY-HUGGINS[21,22,23] theory being well aware of the fact that the idealized molecular model used in developing the theory is quite different from the real DNA molecule.

It can be shown that the coefficient B of equation 2 for many polymer solutions where the entropy of mixing (not the heat of mixing) is the predominant effect is given by:

$$4.\quad B = \frac{(1/2 - \mu)}{\bar{V}_1 d_2^2}$$

where $\bar{V}_1$ is the partial molal volume of solvent, d_2 is the density of the polymer and μ is the well-known interaction parameter of the FLORY-HUGGINS theory. For complete miscibility μ has values less than $1/2$. When μ is $1/2$ or greater, plots of $\overline{\Delta G}$ (partial molal Gibbs free energy) versus μ show a maximum and minimum indicating a separation of the system into two phases—a gel phase and a solvent phase. The critical value, the value at which phase separation occurs, is given by the theory as

$$\mu \text{ critical} = 1/2 \left[1 + \left(\frac{1}{m/m_0} \right)^{1/2} \right]^2$$

where m is the molecular weight of the polymer and m_0 the molecular weight of the solvent. A recent study of a number of water-polar gel systems led to values of μ varying between 0.77 for collagen to 1.15 for silk[24].

The straight lines with positive slope leading to values of μ of $1/2$ or smaller obtained in the 10 experiments cited in Table I, for the $\frac{HC}{\tau}$ versus C plots (shown in Fig. 3) argue against the existence of a network at these low DNA concentrations. A value of μ greater than $1/2$ would lead to an $\frac{HC}{\tau}$ versus C line having a negative slope. It should be mentioned that in some experiments it was noted that as the concentration was increased to values approaching $1 \cdot 10^{-3}$ g/ml there was a tendency for the extrapolated $\frac{HC}{\tau}$ versus C plot to break away from the zero or positive slope straight line to give rise to a negatively sloped line. At these DNA concentrations difficulty is encountered in removing foreign particles and motes which scatter the light from these relatively viscous solutions. Our exploratory observations of the tendency for the $\frac{HC}{\tau}$ versus C plot to change its curvature and take on a negative slope at high concentrations will have to be checked.

To sum up, there appears to be no evidence from the light-scattering experiments for a gel structure of DNA in solution in the concentration range of $0\text{–}7 \cdot 10^{-4}$ g/ml. The light-scattering experiments support the hypothesis that in dilute solutions the DNA molecules are flexible rods capable of a slight amount of coiling.

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EFFECT OF ENZYME DEPOLYMERIZATION ON SCATTERING

Consideration was given to the use of the DNA depolymerizing enzyme[25,26,27] as a means of checking on the amount of light scattering due to impurities. The thought behind this type of experiment was as follows: if, after addition of the depolymerizing enzyme, the turbidity and the dissymmetry fell to some small value one could attribute the original scattering and dissymmetry to the DNA alone. With this in mind a crystallized sample of the enzyme, obtained from the Worthington Biochemical Co. of Freehold, New Jersey, was added under appropriate conditions in the presence of magnesium ions to dilute solutions of DNA. The time changes in dissymmetry and turbidity were then observed.

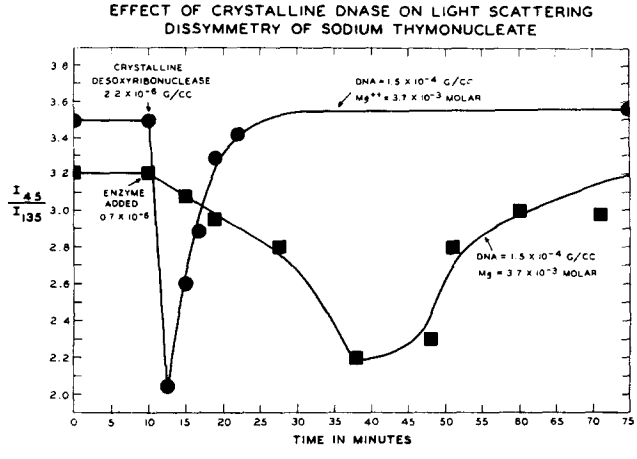

Fig. 6. The above two experiments are typical of 6 sets of measurements.

As shown in Fig. 6 there is a drop in dissymmetry which is followed by a recovery. The time course of the drop and the subsequent recovery are dependent upon the enzyme concentration. It is noted in Fig. 6 that the minimum value of the dissymmetry is close to 2.0. The rate of increase of dissymmetry was proportional to the enzyme concentration. There was no complete disappearance of the turbidity as would be expected if the DNA molecule was degraded to fragments having molecular weights of 10,000 or less. In a half dozen experiments the turbidity dropped to about 25 % of its original value after several additions of enzyme.

The increase in dissymmetry suggested the possibility that micelle formation occurred when the product concentration reached a certain critical value. It was of interest to see if it was possible to determine the particle shape of the aggregate. To this end observations were made in the range of 40°–135°. The observed scattering and the correction factors as a function of time are shown in Fig. 7. These curves did not easily lend themselves to interpretation. In view of the possibility that, in addition to aggregate scattering, there was scattering due to a large enzyme-resistant fraction it seemed advisable that the product mixture be fractionated.

Preliminary experimentation indicated the following: a. 75 % of the products could be dialyzed through a visking bag; b. the dialysate was not precipitatable from a 75 %

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alcohol solution; c. 25 % of the original DNA could be precipitated by 50 % alcohol after prolonged enzyme action. The alcohol precipitable material appeared to be the same substance which was responsible for the residual (25 %) scattering. This material was isolated from the enzyme mixture by alcohol precipitation and centrifugation. It could be obtained in repeated isolation procedures. This material gives the diphenylamine color test[28] characteristic of DNA. It could be shown to be DNA and not RNA, and it analyzed quantitatively (by ultraviolet absorption) as DNA.

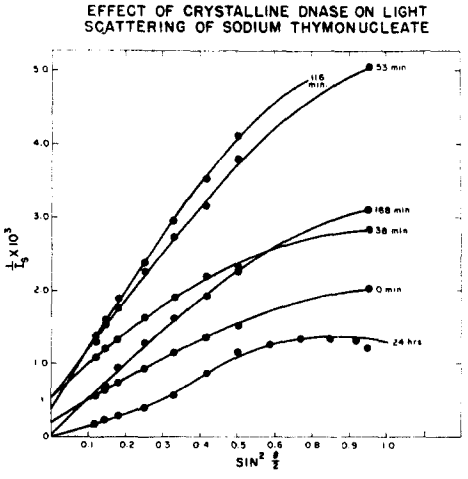

Fig. 7. Reciprocal of I_s, reduced intensity, as a function of the sin $^2\theta/2$ of the angle of observation at different times after addition of the enzyme.

THE ENZYME RESISTANT FRACTION

The light-scattering properties of the enzyme resistant fraction indicate that this material is different from the original DNA. The enzyme resistant fraction isolatable from the equilibrium enzyme mixture has a characteristic light-scattering envelope and dissymmetry. In Fig. 8 there is plotted the scattering envelope of the enzyme resistant fraction. It is noted in Fig. 8 that here, in contrast to Figs. 1 and 2 and the experiments in Table I, the molecular shape is no longer intermediate between a rod and a coil. The enzyme-resistant fraction is well approximated by a rod having a length of 2,646 A.

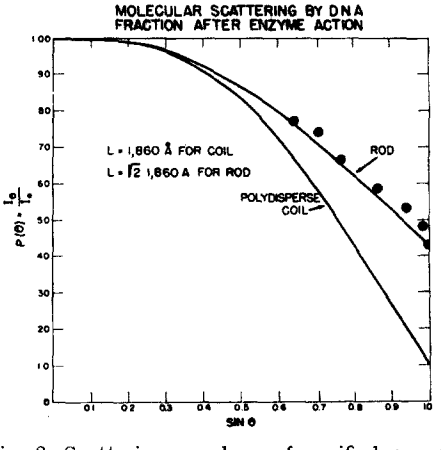

Fig. 8. Scattering envelope of purified enzyme resistant fraction. The polydisperse coil curve was calculated for the FLORY type distribution with parameter $Z = 1$.

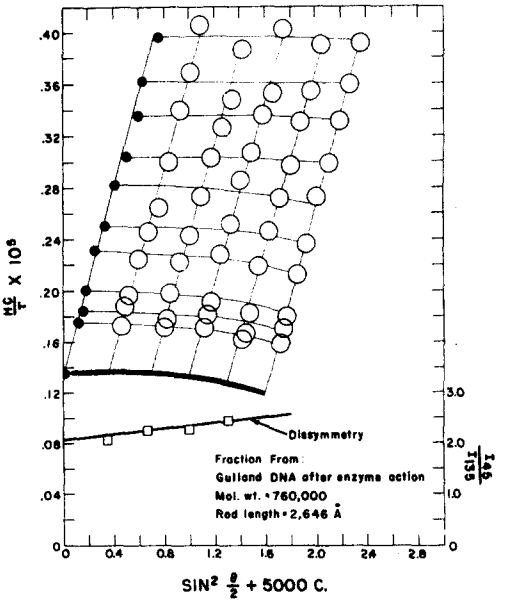

Fig. 9. ZIMM plot of purified enzyme-resistant fraction.

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In Fig. 9 we find the ZIMM plot and dissymmetry of the enzyme-resistant fraction. We note an intrinsic dissymmetry of 2.0 in agreement with the minimum values of Fig. 6. This value is smaller than the theoretical maximum for an infinitely long rod. The curved $\frac{HC}{\tau}$ versus C plot and the high molecular weight—760,000—are a little surprising in view of earlier studies of a DNA core[29].

Since the light-scattering envelope and molecular weight of the enzyme-resistant fraction were markedly different from the undegraded DNA it was of interest to compare the infrared absorption spectrum of the enzyme-resistant fraction with the spectrum of the original DNA. The two spectra are shown in Fig. 10. The strong broad band in the region of 3,300 cm^{-1} is due to the N–H stretching vibration of the amino and imino groups of the purine and pyrimidine residues. The strong bands at 1,660 and 1,750 cm^{-1} are probably due to $C=O$ stretching vibrations of the ring systems. Earlier work[30, 31, 32, 33] has shown that compounds having a phosphoryl (P = O) group have vibrations in the

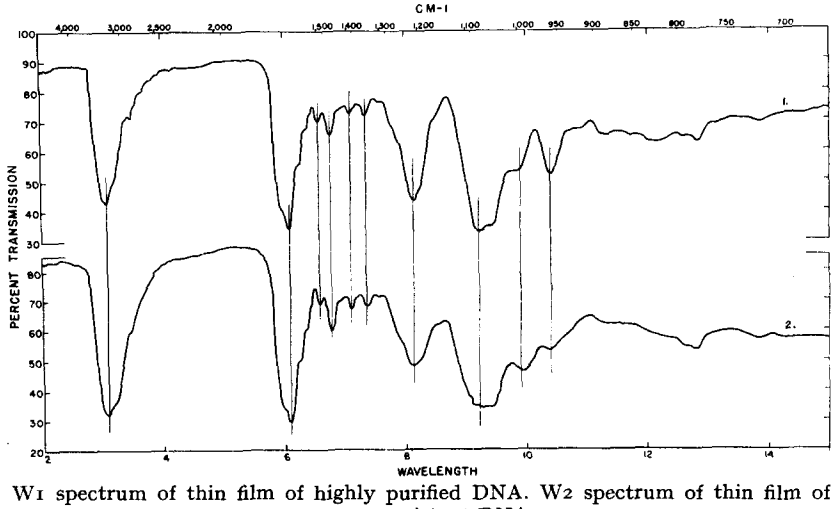

Fig. 10. W1 spectrum of thin film of highly purified DNA. W2 spectrum of thin film of purified enzyme-resistant DNA.

neighbourhood of 1,235 cm^{-1}. The strong band close to 8 microns in Fig. 10 is probably due to this group. The intense broad band at 1,080 cm^{-1} must be associated with the C–O–C vibration of the sugar residues. The two bands between 960 and 1,015 cm^{-1} are believed to be related to two different P–O–C vibrations. The first of these may be visualized as a nucleoside-phosphate ester linkage (1,015 cm^{-1}) and the second as an internucleotide ester linkage (960 cm^{-1}). It is considered of interest that the enzyme-resistant fraction has a relatively small number of intermediate ester (P–O–C) linkages as indicated by the greatly reduced intensity of the band at 960 cm^{-1}.

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SUMMARY

1. The molecular configuration of undegraded DNA is intermediate between that of a rod and a coil. It behaves in light-scattering experiments as though it were a slightly flexible rod.

2. The distance between the ends of this molecule decreases approximately 40% upon the addition of salt as measured by light-scattering and flow birefringence methods.

3. The molecular weight of a highly purified sample of DNA prepared according to the method of GULLAND was $4.51 \pm 0.53 \cdot 10^6$. The molecular length in salt solutions was $6.50 \pm 0.82 \cdot 10^3$ A.

4. An enzyme-resistant fraction was isolated from the DNA after enzyme action. It differed in light scattering and infrared absorption properties from the enzyme susceptible material.

RÉSUMÉ

1. La forme de la molécule non dégradée de ADN est intermédiaire entre celle d'un bâtonnet et celle d'une bobine.

Dans des expériences de dispersion de la lumière, elle se comporte comme un bâtonnet légèrement flexible.

2. A l'aide de méthodes faisant appel à la dispersion de la lumière et à la biréfringence du flux, nous avons trouvé que la distance entre les deux bouts de la molécule diminue d'environ 40% par addition de sel.

3. Le poids moléculaire d'un échantillon très bien purifié d'ADN préparé par la méthode de GULLAND était de $4.51 \pm 0.53 \cdot 10^6$. La longueur de la molécule dans des solutions salines était de $6.5 \pm 0.82 \cdot 10^3$ A.

4. A partir d'ADN traité par un enzyme dépolymérisant nous avons isolé une fraction résistante à cet enzyme. Elle se distinguait de la fraction sensible à l'enzyme par ses propriétés de dispersion et d'absorption dans l'infrarouge.

ZUSAMMENFASSUNG

1. Die Molekelgestalt von unabgebauter DNS ist ein Mittelding zwischen einem Stäbchen und einer Spule. Es verhält sich in Lichtstreuungsversuchen wie ein kaum biegsames Stäbchen.

2. Die Entfernung zwischen den Enden dieses Moleküls nimmt nach dem Hinzufügen von Salz um ungefähr 40% ab, wie mit Methoden der Lichtstreuung und Strömungsdoppelbrechung gemessen wurde.

3. Das Molekulargewicht einer höchst gereinigten, nach der Methode von GULLAND dargestellten Probe DNS war $4.51 \pm 0.53 \cdot 10^6$. Die Moleküllänge in Salzlösungen war $6.50 \pm 0.82 \cdot 10^3$ A.

4. Nach Enzymeinwirkung konnte eine enzymresistente Fraktion aus DNS isoliert werden. Sie unterschied sich durch ihre Eigenschaften bei der Lichtstreuung und Infrarotabsorption von dem enzymempfindlichen Material.

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