MOLECULAR CHARACTERISTICS OF SODIUM DESOXYRIBONUCLEATE

by

JOHN W. ROWEN, MURRAY EDEN AND HERBERT KAHLER

National Cancer Institute, Bethesda, Md. (U.S.A.)*

It has been the purpose of many studies[1-10] on sodium desoxyribonucleate (DNA) to determine molecular parameters in order to define the molecular shape and size. Consideration of this work suggests that a large sample to sample and technique to technique variation is responsible for the enormous range ($0.5 - 20 \cdot 10^6$) of molecular weight values.

We have prepared a high molecular weight sample by the MIRSKY-POLLISTER[11], SEVAG, LACKMAN AND SMOLENS method[12] as modified by GULLAND *et al.*[13] and studied its properties by light scattering, electron microscopy and viscometry. In addition, the viscometric behavior of a second sample prepared by a modified HAMMARSTEN method by GREENSTEIN AND HOYER[14] was studied. This investigation is still incomplete but the measurements thus far obtained are interesting enough to warrant publication at this time. A more detailed account will be submitted upon completion of this investigation.

LIGHT-SCATTERING STUDIES

The intrinsic asymmetries of approximately fifty solutions of the Gulland preparation of varying DNA concentration ($0.40 - 7.0 \cdot 10^{-4}$ g/ml) and salt concentration

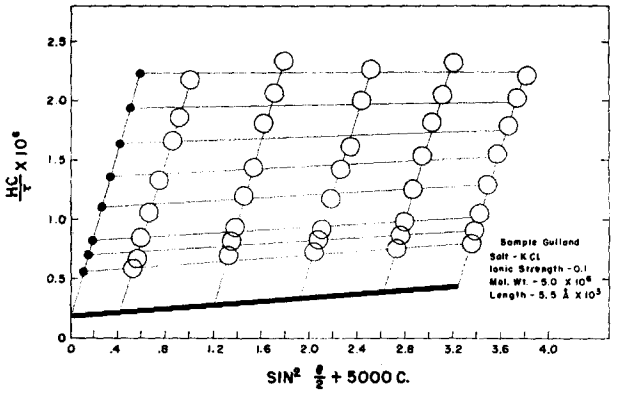

Fig. 1. Typical Zimm plot. The lowest angle of observation of each of the five concentrations, over the range of $1.0 - 7.0 \cdot 10^{-4}$ g/ml, is 40°, the highest point is 100°.

* National Institutes of Health, Public Health Service, Federal Security Agency.

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(0.0–2.5 M) fell between 2.8 and 3.6. The double extrapolation method of ZIMM[15] was found to be applicable to the data. A typical Zimm plot at one ionic strength is shown in Fig. 1. The molecular configuration was intermediate between that of a rod and a coil as shown in Fig. 2. The long thread-like molecules contracted in length from $6.8 \cdot 10^3$ A to $4.5 \cdot 10^3$ A when the ionic strength of the added electrolyte was increased from 0 to $2.0 \cdot 10^{-2}$.

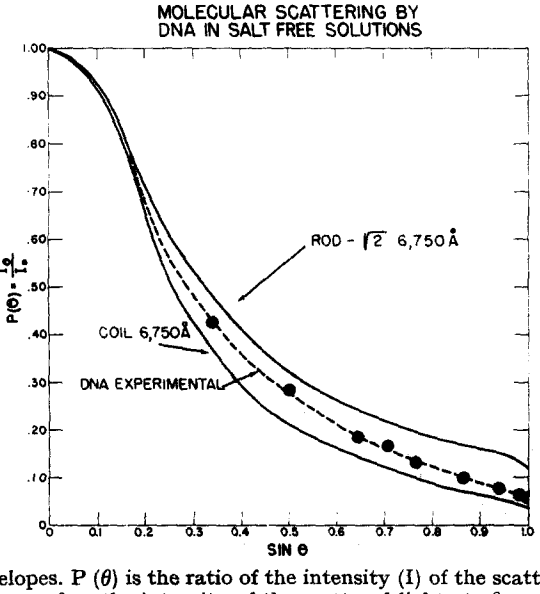

Fig. 2. Scattering envelopes. P (θ) is the ratio of the intensity (I) of the scattered light at any angle θ to the intensity of the scattered light at 0°.

ELECTRON MICROSCOPY STUDIES

In previous electron microscope studies[16,16a] of DNA the diameters measured and reported bore no resemblance to the sizes obtained by other methods and undoubtedly were measurements of multiple filaments. In this investigation a great many electron-micrographs of both of the above-mentioned preparations shadow cast under a variety of conditions with platinum, chromium, and uranium were made. We have succeeded by the use of high resolving power methods and by the proper interpretation of shadow lengths in obtaining measurements on the finest fibers and found them to be in the range of 10–20 A. This value of the molecular diameter is in fair agreement with the values obtained by light-scattering and viscometric methods. Contrary to earlier indications[9,17,18] the molecule appears to be not a stiff rod but one which is best described as a slightly kinked and slightly flexible rod. One of its most striking properties is its tendency to spiral, twist and intertwine with neighboring molecules.

VISCOMETRY STUDIES

The viscometric properties of both samples have been studied and found to be quite comparable. The study of the Gulland preparation is still incomplete. It was found possible to correct the relative viscosities obtained with a Bingham viscometer to zero

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shear rate by two methods which gave essentially identical results. In the first method the pressure was plotted versus shear rate and the limiting slope at zero shear rate was used. In the second method the reciprocal of the product of pressure and time was plotted against pressure and the limiting viscosity was obtained from the extrapolated value of PT[19]. A plot of the specific viscosity versus concentration is shown in Fig. 3. The intrinsic viscosity 5060 ml/g lead to an axial ratio of 330 using SIMHA's equation for a rod[20].

Fair agreement was found in molecular parameters as estimated by the above three methods. The molecular diameter was found to be in the range of 15–24 A, the axial ratio between 330 and 400 and the molecular weight between four and five million ($4.51 - 0.53 \cdot 10^6$).

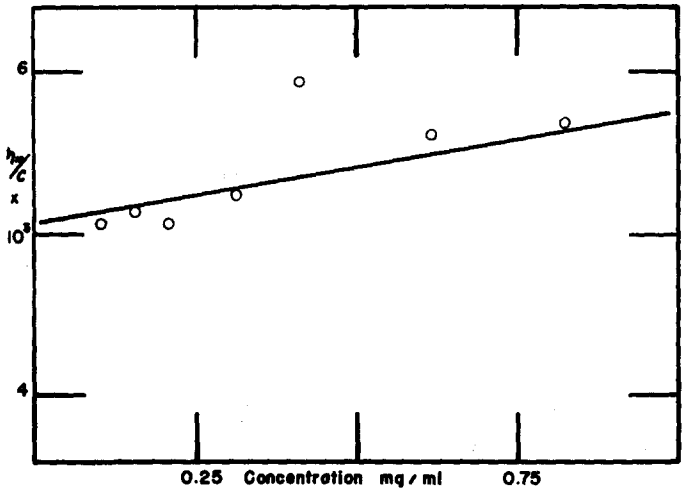

Fig. 3. Intrinsic viscosity determined by extrapolation of the plot of specific viscosity divided by concentrations (mg/ml) *vs.* concentration.

SUMMARY

Studies by three different physical methods on a sample of Na desoxyribonucleate are described.

1. Light-scattering studies indicate that the molecular length decreased from 6800 A for solvent ionic strength zero, to 4500 A for ionic strength 0.02. The molecular weight was found to be $4.5 \cdot 10^6$.

2. Viscosities extrapolated to zero shear rate indicate an axial ratio of 330.

3. Electron microscopic photographs reveal filamentous particles whose diameters are in agreement with values obtained by indirect methods.

RÉSUMÉ

Nous avons étudié un échantillon de désoxynucléate de sodium par trois méthodes physiques différentes.

1. Des expériences avec de la lumière dispersée indiquent que la longueur moléculaire diminue de 6800 A à 4500 A lorsque la force ionique du solvent passe de 0 à 0.2. La valeur trouvée pour le poids moléculaire est de $4.5 \cdot 10^6$.

2. La valeur de la viscosité extrapolée pour une vitesse de cisaillement zéro indique un rapport axial de 330.

3. Des photographies prises au microscope électronique révèlent des particules filamenteuses dont la valeur du diamètre est en accord avec les valeurs trouvées par des méthodes indirectes.

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ZUSAMMENFASSUNG

Untersuchungen einer Probe Natriumdesoxyribonucleat mit drei verschiedenen physikalischen Methoden werden beschrieben.

1. Untersuchungen im Streulicht zeigen, dass die Molekülläge von 6800 A in Lösungen mit der Ionenstärke 0 abnimmt auf 4500 A bei Ionenstärke 0.02. Das Molekulargewicht wurde zu $4.5 \cdot 10^6$ gefunden.

2. Die für Schergeschwindigkeit Null extrapolierte Viskosität zeigt ein Achsenverhältnis von 330 an.

3. Aufnahmen mit dem Elektronenmikroskop zeigen faserige Teilchen, deren Durchmesser in Übereinstimmung sind mit den durch indirekte Methoden erhaltenen Werten.

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