SURFACE FILMS OF DESOXYRIBONUCLEIC ACID

by

THOMAS W. JAMES* AND DANIEL MAZIA**

Department of Zoology, University of California, Berkeley, Calif. (U.S.A.)

High polymer desoxyribonucleic acid (DNA) isolated from calf thymus or from chicken erythrocytes can be induced to spread as a film on the surface of a 2*M* sodium chloride solution. The thickness of the film is within the range of nucleotide dimensions, and the measurements reported below may contribute to the analysis of the structure of DNA.

METHODS

In general, conventional methods of surface chemistry were used to explore the properties of DNA films. A Langmuir trough (Central Scientific Co., Chicago), provided with a deep well, and a vertical-pull surface balance were used to obtain the force-area curves. The DNA was isolated by the method of MIRSKY AND POLLISTER[1]. It was highly polymerized, forming a fibrous precipitate with protamine[2] and having an optical density of 20.6 (0.1% solution, one cm path) at its 260 millimicron peak in the ultraviolet[3]. The concentration of the aqueous solution of DNA which was applied to the clean sodium chloride surfaces was 0.1 mg/ml. Higher concentrations left unspread patches, whereas lower concentrations produced films whose thickness corresponded to those formed from the 0.1 mg/ml solution. The aqueous solution was slowly deposited on the surface by a controlled pipette. However, the total amount of DNA applied did not remain at the surface, therefore two direct methods were employed for determining the amount which was spread.

The first method employed a plate upon which a given fraction of the monolayer was deposited at a constant pressure using the method of LANGMUIR AND BLODGETT[4]. The deposited film was then dissolved off in a definite amount of water and its concentration was determined by use of a Beckmann spectrophotometer. Fortunately, the high absorption coefficient of DNA made such determinations possible. Knowing the fraction of the surface which such a dipping represented, the total surface concentration could be determined and was used to adjust the relative position of the abscissa of the force-area curve. The second method used to determine the amount of DNA remaining on the surface was to strip the film completely from the surface after it had been compressed above the collapse pressure (see Fig. 1). This can be accomplished if the pH of the subphase is lowered to about pH 3, for at this pH the film becomes so coherent at small areas that it can be lifted from the surface in the form of elastic fibers. By the simple observation of talc particles previously deposited on the film, one can easily convince oneself that the film is being collected into the fiber as it is being withdrawn. In fact, with care the entire surface can be withdrawn as a single fiber. The DNA may then be dissolved and determined spectrophotometrically.

For the direct determination of film thickness, an ellipsometer[5], which was calibrated against barium stearate monolayers, was constructed. DNA films were deposited over the barium stearate step plate that had previously been prepared on a stainless steel slide. The step plate was immersed in the subphase prior to the spreading of the DNA film. It was withdrawn while the film was held at a pressure of 5 dynes per cm. The technique of measuring film thickness was similar to that described by ROTHEN[6].

For the study of pH effects, an apparatus was devised which permitted the control of the pH of the subphase without disturbing the film, thus making it possible to follow the relation between pH and surface pressure on a single film. The input and output of a circulating pump constructed

* Research Fellow, U.S. Public Health Service.

** This work was supported in part by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

References p. 370.

of glass and polyethylene were connected to opposite ends of the trough. Effective mixing was achieved by introducing the liquid returning from the pump through a perforated tube extending across the width of the trough. The dipping-plate and a glass electrode system were introduced at the outflow end. With this system it proved to be possible to circulate the subphase at the rate of one liter per minute without loss of surface pressure. As HCl or NaOH solutions were slowly titrated into the system, the circulation quickly brought it to constant pH, and the film pressures were recorded.

RESULTS

A typical force-area curve for DNA at pH 7 is given in Fig. 1. The extrapolated straight portion of the curve intersects the area axis at 0.280 square meters per milligram. Using a density value of 1.65[6], the thickness of the film can be calculated. Values found by this method in eight independent experiments are given in Table I. These values are for DNA films spread on a subphase at pH 7. The mean value of 22.3 A is 50 per cent higher than the greatest nucleotide dimension, 13.8A[6].

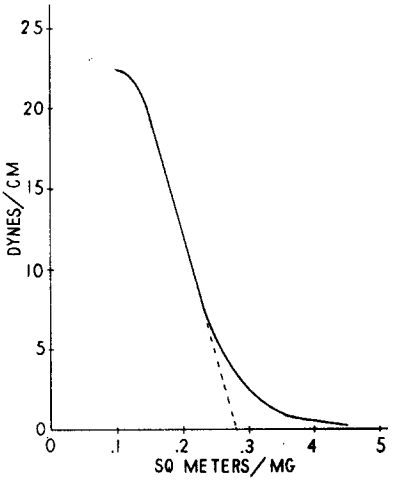

Fig. 1. Force-area curve for DNA films spread over 2 *M* NaCl at pH 7. The amount of DNA present in a given film was determined by analysis as described in text.

TABLE I

DNA FILM THICKNESS

(In A)

Volume/Surface Area Measurements	*Ellipsometer Measurements*
21.7	21.9
21.1	19.0
24.6	21.6
23.0	22.2
17.0	21.9
21.0*	
22.6*	
28.0*	
Mean = 22.3 A	Mean = 21.6 A

* Measurements by dipping-slide method. Other values in this column obtained by recovering film as a fiber.

The measurements with the ellipsometer served as a check on the values for film thickness calculated from the spectrophotometric determinations. These are given in the second half of Table 1. The average value obtained at pH 7 is 21.6 A, which is in good agreement with the values obtained from the force-area curves. The concordance of the results obtained by the several methods suggests that the value of about 22 A for the thickness of DNA films at pH 7 may have some real significance with respect to the structure of the DNA molecule.

The mechanism of surface-spreading of DNA is not clear, since the molecule as commonly formulated would not seem to have a marked hydrophilic-hydrophobic axis. Since the nucleotide units have a distinct acid-base axis, some clue as to the orientation or aggregation of the polymer molecules might be obtained by investigating the pH dependence of the physical properties of the films. Using the method described above, the relation between surface pressure and pH of subphase was obtained for films initially spread at pH 7. Such a pH-pressure curve is shown in Fig. 2, the arrows representing the direction of the titration. The curve is relative in the sense that the selection of the

References p. 370.

initial pressure (10 dynes/cm) was arbitrary. There is clearly a tendency for the film to expand as the pH is lowered. Exposure to acid pH brings about some irreversible change that is described by the pressure-pH relationship on the returning phase of the pH cycle shown in Fig. 2. One consequence of this irreversible alteration was that the film became unstable at pH 7, and thus the observations could no longer be extended into the alkaline range. Moreover, the alteration was such that we could not successfully deposit the films on a solid surface after return to pH 7, and therefore were unable to compare the thickness before and after exposure to acid.

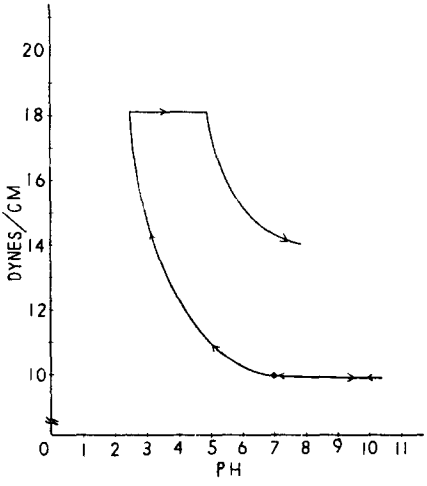

Fig. 2. Variation of pressure of a DNA film, at constant area, with alteration of pH of the underlying solution. Film was initially spread at pH 7 (●) and arrows indicate course of pH changes.

DISCUSSION

The film thickness of approximately 22 A, obtained by two essentially independent methods, may have some bearing on the details of the structure of DNA or its aggregates. The value is just 50% greater than that expected from a simple stack of nucleotides. It corresponds fairly well to the limiting spacing observed by RILEY AND OSTER[7] in X-ray diffraction studies of concentrated DNA solutions. The existing data do not permit a choice between several alternative structures that would account for the thickness. 1. We may be measuring the average thickness of DNA micelles such as were postulated by GULLAND AND JORDAN[8]. 2. The measured thickness may be the result of a spiral structure such as has been considered by RILEY AND OSTER in their analysis of the X-ray diffraction data. 3. The individual nucleotides of a single polymer change may be capable of rotation about the phosphate backbone. Experiments with molecular models show that the thickness predicted from a chain of freely rotatable nucleotides would be close to the 22 A obtained in our measurements.

The fact that the thickness measured is less than that of two nucleotides suggests very strongly that, regardless of whether any of the above interpretations is correct, the DNA polymer chains lie parallel to the surface, and that the films are truly monomolecular.

Since DNA is highly soluble in water and in salt solutions, it is not entirely clear how stable films yielding reproducible data can be formed at all. Presumably the films could be prepared over 2 *M* NaCl and not over water because the DNA solution in water which was used in spreading is surface active relative to the strong salt solution, and therefore would form a surface layer of DNA solution over salt solution. But this does not explain why the DNA does not ultimately dissolve in the underlying NaCl solution. The observed fact was that our films were stable for many hours, or as long as they were under observation. The observations represented in Fig. 2 show a tendency for the film to expand as pH was lowered. One possible explanation is that the basic groups of the DNA are predominantly affected[9]. The fact that the pressure does not return to its original value at pH 7 may be interpretable as an irreversible modification involving the

References p. 370.

basic groups, for which the disaggregation effects postulated by GULLAND AND JORDAN[8] might serve as a model. Presumably other hypotheses would account equally well for the decrease in thickness at low pH, and what is now required is a study of the pH effect on the thickness either by measuring area changes at constant pressure or by finding some means of depositing the modified films on solid surfaces for thickness measurements.

SUMMARY

High polymer desoxyribonucleic acid may be spread over 2 *M* NaCl at pH 7, the material being deposited on the surface from dilute aqueous solution. The thickness of the films has been estimated by extrapolation of force-area curves of films of known initial concentration and by direct optical measurement of the thickness of films deposited on solid surfaces. Both methods yield a value of approximately 22 A for the thickness of the DNA films. The thickness tends to decrease as the pH of the underlying solution is lowered, and the decrease is associated with an irreversible change in the system. The possible bearing of these observations on the analysis of the structure of DNA is discussed.

RÉSUMÉ

Lorsqu'un acide désoxyribonucléique haut-polymère en solution aqueuse diluée est porté à la surface d'une solution de NaCl 2 *M* à pH 7, il peut former un film. Nous avons évalué l'épaisseur de ces films par extrapolation des courbes force-aire de films de concentration initiale connue et par mesure optique directe de l'épaisseur de films étendus sur des surfaces solides. Par ces deux méthodes, nous avons obtenu, pour l'épaisseur de films d'ADN, une valeur d'approximativement 22 A. L'épaisseur tend à diminuer lorsque la pH de la solution sous-jacente diminue et cette diminution d'épaisseur est accompagnée d'un changement irréversible du système. Nous avons discuté la portée possible de ces observations sur l'analyse de la structure de l'ADN.

ZUSAMMENFASSUNG

Hochpolymere, aus einer verdünnten wässrigen Lösung auf die Oberfläche aufgebrachte Desoxyribonukleinsäure kann sich auf 2 *M* NaCl bei pH 7 ausbreiten. Die Dicke des Films wurde durch Extrapolation der Kraft- Flächeninhalt-Kurven von Filmen bekannter Anfangskonzentration und durch direkte optische Messung der Dicke von auf festen Oberflächen sitzenden Filmen bestimmt. Beide Methoden ergaben einen Wert von ungefähr 22 A für die Dicke der DNS-Filme. Die Dicke des Films neigt dazu abzunehmen, wenn das pH der darunterliegenden Lösung geringer wird und dieses Abnehmen ist mit einer irreversiblen Veränderung des Systems verbunden. Die mögliche Auswirkung dieser Beobachtungen auf die Strukturanalyse der DNS wird besprochen.

REFERENCES

1 A. E. MIRSKY AND A. W. POLLISTER, *J. Gen. Physiol.*, 30 (1946) 117.
2 D. MAZIA, *Growth* (Symposium supplement), 9 (1949) 5.
3 M. KUNITZ, *J. Gen. Physiol.*, 33 (1950) 349.
4 I. LANGMUIR AND K. BLODGETT, *J. Am. Chem. Soc.*, 56 (1934) 495.
5 A. ROTHEN, *Rev. Sci. Instrum.*, 16 (1945) 26.
6 W. T. ASTBURY, *Symp. Soc. Exptl. Biol.*, 1 (1947) 66.
7 D. P. RILEY AND G. OSTER, *Biochim. Biophys. Acta*, 7 (1951) 526.
8 J. M. GULLAND AND D. O. JORDAN, *Symp. Soc. Exptl. Biol.*, 1 (1947) 56.
9 N. K. ADAM, *The Physics and Chemistry of Surfaces*, Oxford University Press, London, 1941.

Received August 30th, 1952