PREPARATION OF ORIENTED DNA IN LARGE AMOUNTS x)

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Received May 31, 1963

For many physicochemical investigations of macromolecules, it is desirable to utilize samples with a high degree of molecular orientation. In the case of DNA, four different methods of orientation have been used.

In most of their work on X-ray diffraction of DNA, Wilkins et al. used fibres 50 to 100 μ in diameter made by slowly withdrawing a glass point from a sticky DNA gel (Wilkins et al., 1951).

Thin films for birefringence and dichroism studies have been prepared by shearing a gel between a coverslip and slide (A review is given by Seeds, 1953) or by stroking the gel with a spatula (Falk et al., 1963).

In the liquid state, orientation of DNA has been achieved by applying an electrical field in short pulses (Norman and Field, 1957; Dvorkin, 1961; Allais, 1962; Ingram and Jerrard, 1962).

Another well-known method for orientation in polymer solutions is the shearing of the solution between two cylinders, one of which rotates (Goldstein and Reichmann, 1954; Barbu and Joly, 1956).

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x) This work was supported by PHS Research Grant C-5799 from the National Cancer Institute, Public Health Service, Bethesda.

In this connection, reference may be made to a method by Spitkovsky which gives oriented nucleoprotein (Spitkovsky, 1955). By letting a drop of nucleoprotein solution fall through a precipitating liquid, a tail with a certain degree of orientation is formed behind the falling drop.

None of the methods mentioned, however, permits physicochemical investigations where solid or gelatinous samples of oriented DNA in large amounts and having a uniform orientation are needed. For that reason we believe that the method here described for preparation of oriented DNA will meet a pressing necessity and make possible semiconductivity measurements, NMR- and ESR-measurements, etc. on oriented DNA. The method gives oriented DNA in homogeneous films of any desired dimensions. It utilizes simultaneous precipitation and stretching of a DNA solution which flows continuously from a capillary into a precipitating liquid. The stretching is performed by continuously winding the DNA thread formed onto a rotating cylinder. During the rotation, the cylinder is given a slow axial movement back and forth within desired limits and the thread, guided by a fixed V-shaped thread guide, is wound in layers on the cylinder. When the desired thickness is achieved, the cylinder with the DNA still on it is dried in air at the desired relative humidity, giving an apparently homogeneous film.

The first stage of the preparation thus involves the wet spinning of a DNA solution which, from the slightly related process of viscose spinning, is known to give orientation (Krebs, 1942). The second stage involves the agglomeration of the different DNA threads into a homogeneous mass during the drying.

The apparatus consists of a precision pump for the feeding of the DNA solution, a thermostated precipitation tower at the top of which the capillary enters into the precipitating liquid,

a vessel with a rotating cylinder immersed in the precipitating liquid, a thread guide directing the thread onto the rotating cylinder and auxilliary equipment for the rotation and the axial movement of the cylinder.

In the experiments performed, ethyl alcohol has been utilized as precipitating liquid. The following values of the variables of the process have been used:

The concentration of the DNA solution	1-3 mg/ml
The diameter of the capillary	0,1-0,4 mm
The alcohol concentration	75-85 %
The electrolyte concentration	0,025-0,1 M NaCl (or LiCl)
The feeding of the DNA solution	0,05-6 ml/h
The speed of the rotating cylinder (10 cm diam.)	up to 10 rpm
The temperature	25°C and -5°C

In fibre spinning, the deformation ratio S (Ziabicki and Kedzierska, 1959) is defined as the ratio between the speed at which the thread is wound up on the cylinder and the speed at which the DNA solution flows out of the capillary. The maximum deformation ratio utilized up to now without breakage of the DNA thread is about S = 150, which, under the circumstances of the experiment, corresponds to an estimated DNA thread diameter of about 1 μ.

If desired the DNA film can be prepared directly on glass or quartz slides by fastening the latter onto the surface of the rotating cylinder. The DNA threads then are deposited directly on the slides giving an oriented film on drying.

Test of the oriented DNA

All the preparations made by the method show a strong birefringence in the visible spectrum which indicates a good

orientation (Ziabicki and Kedzierska, 1959). From earlier investigations it is well-known that oriented DNA samples also show negative dichroism in the ultraviolet spectrum. This means that the dichroic ratio R is smaller than one, $R=\frac{A_{11}}{A_{\perp}} < 1$, where A_{11} and $A_{\perp}$ are the absorbance of the band with the electric vector of radiation polarized parallel and perpendicular, respectively, to the direction of the molecular orientation. The negative dichroism is understandable by the fact that the transition moments lie in the plane of the bases. In a recent study, Falk et al. (Falk et al. 1963) correlated the dichroic ratio at 2700 Å of oriented NaDNA film at various r.h. with the DNA structure. They found a smallest value R=0,31 above 85% r.h., where the B-form with the base-pairs stacked perpendicular to the axis of the helix exists. A similar study on oriented NaDNA film was made by Wilkins and Seeds (Seeds, 1953) and their preparation gave an even lower value, R(2700Å)=0,21 at 90% r.h.. A lowest value of R(2600Å)=0,31 has been found for fibres (Thorell and Ruch, 1951).

To test our method, we made a preparation of LiDNA (commercial NaDNA dialyzed in 3 M LiCl) on a quartz slide utilizing a deformation ratio during the spinning of S=45. The deposit on the slide was formed from about 2000 parallel DNA threads giving a film with the dimensions 13 x 30 mm^2 holding 0,0254 mg DNA/cm^2 as calculated from the absorbance of the original DNA solution. The dichroism measurements were made on a Zeiss PMQ II spectrophotometer supplied with a Glan prism outside the exit slit. Measurement was also made with unpolarized light which, according to theory, if the orientation is good, should give an absorbance of $A=-^{10}\log \frac{10^{-A_{11}}+10^{-A_{\perp}}}{2}$. This corresponds to an arithmetic mean value of the transmissions. The result of the measurements at 2700Å is shown in the table. The r.h. was 93 %.

$A_{\perp}$	A_{11}	A(calc.)	A(exp.)	R
0,96	0,27	0,49	0,52	0,28

The figures indicate that a high degree of orientation is achieved.

As DNA is the carrier of genetic information it is important that the transforming activity is not destroyed by the method of orientation. To test this, a solution of Bacillus subtilus-DNA, later checked for transforming activity of two different markers in the strain SB1, was utilized for spinning in the apparatus, various deformation ratios being employed. Samples were taken directly from the rotating cylinder during the spinning and after five days of drying at 75% r.h. and at a temperature of -5°C. The transforming activity of the samples was tested and the result indicates that this activity is not destroyed by moderate stretching and drying at 75% r.h..

The preparation of oriented DNA in large amounts, with a uniform and reproducible orientation while retaining of the transforming activity, makes possible many experiments of biochemical and physicochemical interest. We are thus preparing semiconductivity measurements, NMR- and ESR-measurements on oriented DNA. By introducing pressure as another variable in the measurements, we hope to be able to elucidate certain pressure effects.

A fuller investigation of the influence of the variables of the orientation method is under preparation. If possible the method will also be applied to some other biopolymers.

Acknowledgements

The author wishes to express his gratitude to Dr. C.-G. Rédén, who initiated this research, for many valuable discussions and for excellent working facilities. Thanks are further due to Dr. T. Lindahl for the transforming activity measurements.

References

Allais, M.-L., J.Chim.Phys., 59,873 (1962)
Barbu, E. and M. Joly, J.Chim.Phys., 53,951 (1956)
Dvorkin, G.A., Biofizika, 6,403 (1961)
Falk, M., K.A. Hartman,Jr., and R.C. Lord, J.Am.Chem.Soc., 85,391 (1963)
Goldstein, M. and M.E. Reichmann, J.Am.Chem.Soc., 76,3337 (1954)
Ingram, P. and H.G. Jerrard, Nature, 196,57 (1962)
Krebs, G., Kolloid-Z., 98,200 (1942)
Norman, A. and J.A. Field, Jr., Arch.Biochem.Biophys., 71,170 (1957)
Seeds, W.E., Progress in Biophysics, 3,27 (1953).Eds. J.A.V. Butler and J.T. Randall, Pergamon Press, London
Spitkovsky, D.M., Biochimija 20,566 (1955)
Thorell , B. and F. Ruch, Nature, 167,815 (1951)
Wilkins, M.H.F., R.G. Gosling and W.E. Seeds, Nature, 167,759 (1951)
Ziabicki, A. and K. Kedzierska, J.Appl.Polymer Sci., 2:4,14 (1959)