THE ORIENTATION OF THE RIBONUCLEIC ACID WITHIN YEAST RIBOSOMES DETERMINED ELECTRO-OPTICALLY

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ABSTRACT Electro-optical birefringence and ultraviolet dichroism have been recorded from solutions of 80S yeast ribosomes. The sense of both of these effects is consistent with an orientation of the ribonucleic acid within a ribosome such that the plane of the purine and pyrimidine bases is predominantly parallel to the electric axis of the ribosome.

1. INTRODUCTION

Although ribosomes have been studied intensively for the past 8 or 10 years, whether or not they have any definite secondary or tertiary structure has not been known for certain. The electron microscope has shown their nearly spherical form and the fact that, in contradistinction to many spherical viruses of similar size and composition, there is no clear spatial separation of nucleic acid and protein within ribosomes (Huxley and Zubay, 1960). Using the analytical ultracentrifuge, many investigators have shown a variety of subunits within the 70 or 80S ribosome, but units smaller than the characteristic $\frac{1}{3}$ and $\frac{2}{3}$ fragments are subject to some disagreement at the present time. From x-ray diffraction patterns given by gels of unoriented ribosomes, only one spacing at 45.5 A has been found which could be ascribed to internal structure (Langridge and Holmes, 1962).

In general, it is only possible to learn details of spatial structure when the object being studied can be oriented. If the object is not found oriented by nature, then it must be oriented artificially. If the object is asymmetric, orientation can be produced by one or another form of shearing stress. If the object is nearly or exactly spherical, shearing stresses will have no orienting effect and some other orienting force must be employed. One such force which has been found useful is an applied electrical field. In the hands of Benoît (1951) and O'Konski (1960) this technique has been highly developed. The orientation of a variety of macromolecules in solution (tobacco mosaic virus, DNA, serum albumin, etc.) has been observed by means of the birefringence thus induced within the solution. Recently, Dvorkin (1960, 1961) has recorded changes in the absorption of linearly polarized ultra-

violet light by solutions of DNA and RNA during the application of electric fields (electric ultraviolet dichroism) and thus learned more directly the orientation of the purine and pyrimidine bases with respect to the electrical axis of these molecules.

The present work has applied these electro-optical methods to the study of 80S ribosomes from yeast. The conclusions here drawn are that these ribosomes show positive birefringence and are dichroic in electric fields, and that both these electro-optic effects are consistent with a definite spatial organization of the nucleic acid within ribosomes such that the plane of the bases is predominantly parallel to the ribosomes' electrical axis.

2. APPARATUS AND MATERIALS

(*a*) *Apparatus.* A hard tube pulser, capable of applying square electric pulses, singly or repetitively, of both polarities, of several kilovolts with currents up to 10 amps, and of 0.1 or 1 msec. duration across solutions containing 10^{-3} or 10^{-4} M Mg^{++} ions was constructed for these studies by G. F. Vanderschmidt and R. C. Abbe of the Lion Research Corporation, Cambridge, Mass. The pulses were applied to 1 cm^2 platinum electrodes, spaced 0.2 cm apart, which fitted into a 1 cm path-length quartz absorption cell, after the design of O'Konski. The cell was held in a brass holder through which ice water was circulated. The light source was a General Electric UA-2 250 watt Hg arc. It was operated on DC either by means of a rectifier or by 10 12-volt automobile batteries connected in series (to give a truly ripple-free illumination). A Farrand grating monochromator selected a suitable Hg line. This light was polarized either by a quartz disc which had been covered with the dichroic polarizing material, PL40 UV, by the Polacoat Corp., Blue Ash, Ohio, for ultraviolet measurements, or by sheets of Polaroid or a Glan-Thompson prism for work in the visible. The light leaving the cell fell upon an RCA 1P28 photomultiplier whose output, after suitable amplification, was observed and photographed on the screen of a DuMont 411 oscilloscope. This oscilloscope afforded simultaneous presentation of the electric field and the light signal. The time constant of the detection system was about 5 μsec. A block diagram of the apparatus is given in Fig. 1. Details of the electronics may be obtained from Dr. Vanderschmidt.

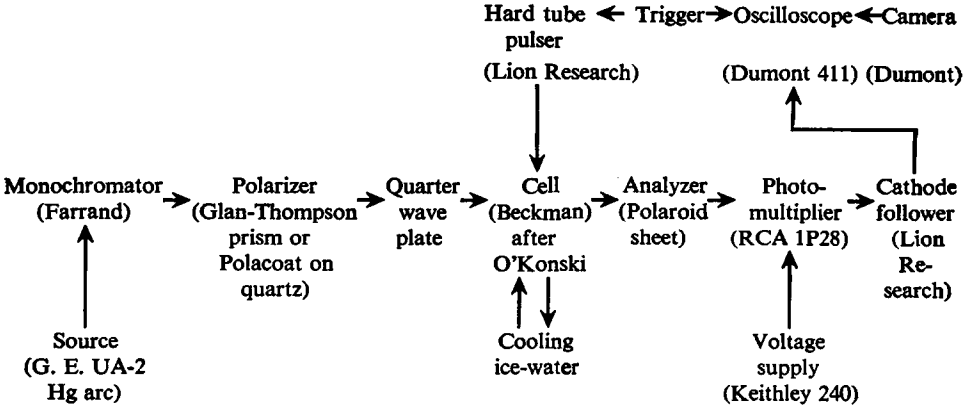

FIGURE 1 Apparatus for transient electric birefringence and dichroism. The quarter-wave plate and analyzer are removed for measurements of dichroism.

(*b*) *Ribosomes.* 80S yeast ribosomes were prepared in 10^{-4} M Mg acetate, brought to pH 5.0 with 1 M acetic acid, from cells in logarithmic growth on a synthetic medium by the methods described before (Morgan, Cunningham, and Greenspan, 1963). The washed ribosomes were usually quickly frozen, stored in this state, and thawed just before use. Examination in the analytical ultracentrifuge and by electron microscopy (kindly performed for us by Dr. Elizabeth Maclean) showed that these preparations were over 90 per cent 80S particles. Similar results were obtained when a buffer containing 10^{-3} M Tris-HCl, pH 7.5 and 10^{-3} M $MgSO_4$ was used.

3. MEASUREMENTS

1. *Birefringence*

(*a*) *Optics.* The sample cell was placed between polarizer and analyzer, whose directions of transmission were at 90° to each other and at 45° to the horizontal axis of the electric field. Following the suggestion of O'Konski and Zimm

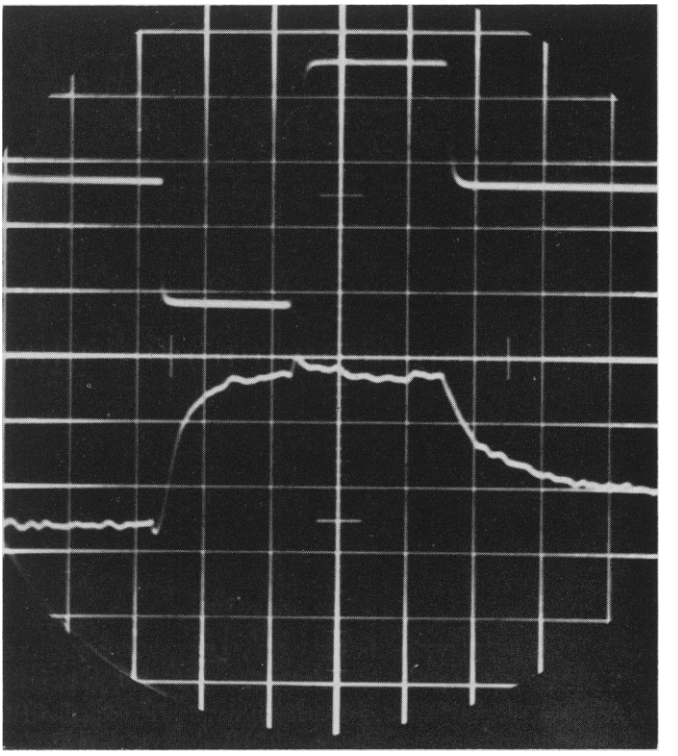

FIGURE 2 Ribosomal electric birefringence. The upper trace is proportional to the electric field (1 cm = 5 kv/cm). The lower trace is proportional to the transmitted light intensity, a greater intensity giving a downward deflection. Conditions: concentration 0.5 mg/ml in 10^{-1} M MgAc, pH 5.0; temperature 1°C; pulse duration 0.1 msec. each polarity; the $\lambda/4$ plate has its slow axis perpendicular to the field; wave length 436 mμ; 0.20 cm gap.

(1950), a rotatable quarter-wave plate made of cleaved mica by the Baird-Atomic Co. was placed between polarizer and cell so that its slow axis could be either parallel or perpendicular to the field. The plate had a retardation of 90 mμ, and the Hg lines at 366 and 436 mμ were used. With such an arrangement, when the slow axes of the λ/4 plate and the sample coincide, more light passes the analyzer; when they oppose each other, less light. Thus the sign of the birefringence is directly determined. Under these conditions the retardation of the sample, δ, in radians is $\sin^{-1} 2\ \Delta I/I_{max}$, where ΔI is the change in transmitted light due to the impressed birefringence, and I_{max} is the transmitted light when the axes of polarizer, λ/4 plate and analyzer are all parallel, and the sample is in place. The light intensity was taken to be proportional to the voltage on the oscilloscope. Without the λ/4 plate, the sample's retardation is $\cos^{-1} (1-2\ \Delta I/I_{max})$.

(*b*) *Results.* Fig.2 shows a typical oscillogram of ribosomal birefringence and Fig. 3 shows the variation of this quantity with field strength and with con-

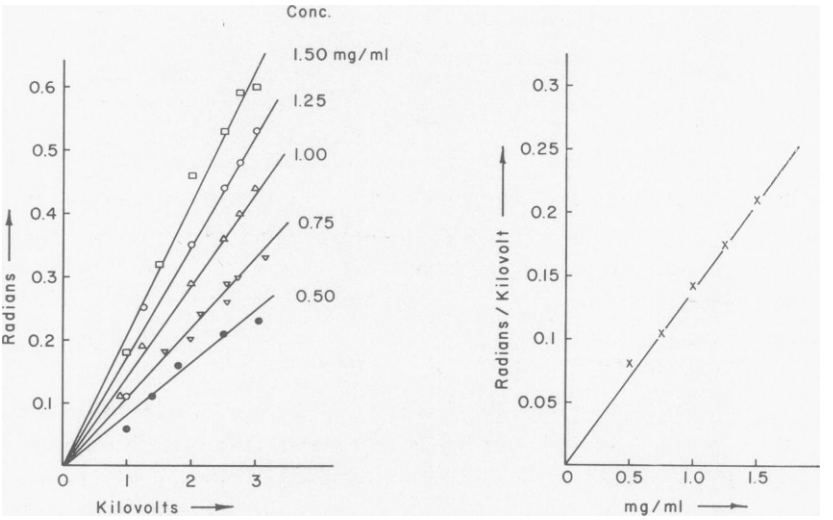

FIGURE 3*a*

FIGURE 3*b*

FIGURE 3 Dependence of ribosomal electric birefringence on (*a*) electric field and (*b*) concentration. Conditions: buffer 10^{-4} M MgAc, pH 5.5; temperature, 1°C; pulses 0.1 msec., one polarity only; λ/4 plate removed; wave length 366 mμ; 0.25 cm gap.

centration. The dependence appears to be linear in both cases and allows the whole data to be summarized by a single "Kerr constant" of $3.5 \cdot 10^{-5}$ radians/(volt/cm)/(mg/ml). The birefringence is positive; *i.e.*, the greater refractive index of the solution is parallel to the electric field. Fig. 2 also demonstrates that when the polarity of the field is abruptly changed, there is little or no change in the birefringence. O'Konski and Haltner (1957) have stated that such behavior implies that induced dipoles are responsible for the orientation causing the birefringence

seen here. In experiments not shown in Fig. 3 it was found that a field strength of 12 to 14 kv/cm appeared to saturate this birefringence.

The decay of the birefringence is plotted in Fig. 4. The data cannot be fitted by a single straight line. On the line of greatest slope, the birefringence decays to $1/e$ of its original value in 60 to 80 μsec. If Stokes law for spheres were obeyed, this relaxation time would correspond to a sphere of radius about 230 A. It is not at all

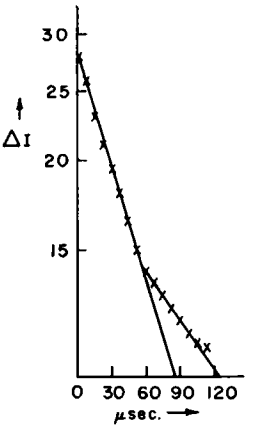

FIGURE 4 Relaxation of ribosomal electric birefringence. ΔI is plotted against time for the pulse shown in Fig. 2.

certain that ribosomes do obey Stokes law. But in any case, the relaxation time is consistent with an object of the size of ribosomes and indicates that, in all probability, it is the whole ribosome, and not some part of it, which rotates when the electric field is removed.

2. *Ultraviolet Dichroism*

(*a*) *Optics.* The optical arrangement needed to observe dichroism is simpler than that needed for birefringence: between the monochromator and the phototube only the polarizer and the cell are interposed. A change of light intensity due to the field which reverses its sense on rotating the polarizer through 90° constitutes dichroism. If more light is passed when the electric vector of the light is parallel to the impressed field, then the transition moment of the absorbing chromophore must be predominantly perpendicular to the field. No greater precision concerning the orientation of this moment can be obtained without some independent measurements of the degree of molecular orientation produced by the field. The dichroic ratio, $R = OD_{\parallel}/OD_{\perp}$ is determined by measuring the light intensity with buffer in the cell (I_0) and then the intensity with sample during the applied field and with polarizer first parallel and then perpendicular to the field. That is $R = [\log (I_0/I_{\parallel})]/[\log (I_0/I_{\perp})]$, and is thus independent of optical path length or concentration, assuming Beer's law is valid. Suitable concentrations of ribosomes were in the neighborhood of 0.03 mg/ml, corresponding to an optical density at 260 mμ

of about 0.5 for the unoriented material. Measurements were made using the 254 Hg line.

(*b*) *Results.* Fig. 5 shows an oscillogram of ribosomal ultraviolet dichroism. The increase in noise is due chiefly to the low level of transmitted light. When the electric vector of the incident light is perpendicular to the field, an increase in transmitted light is seen. Hence the transition moments of the absorbing groups are predominantly parallel to the field. The maximum dichroic ratio observed is about 1.10. For comparison, we have observed ratios of 1.5 with solutions of tobacco mosaic virus (kindly given us by Dr. Robert Langridge), and Dvorkin (1960) has recorded electro-optically dichroic ratios of 1.2 from solutions of TMV ribonucleic acid. With ribosomes, fields greater than about 6 kv/cm do not produce greater dichroism. Why this effect is saturated at about half the field strength needed to saturate birefringence is not understood.

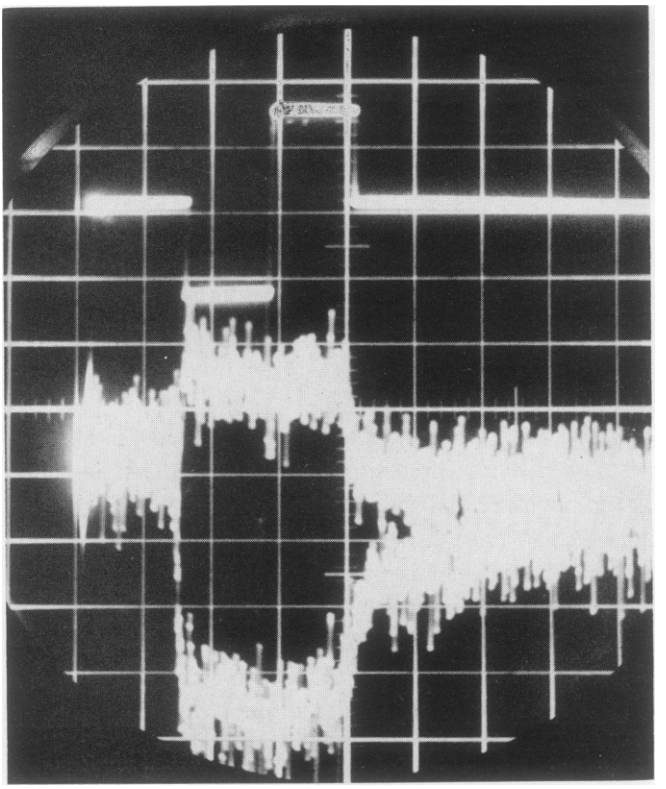

FIGURE 5 Ribosomal electric ultraviolet dichroism. The lower pair of traces are proportional to the light transmitted when the electric vector is parallel to the field (upper trace) and perpendicular to the field (lower trace). Conditions: concentration 0.05 mg/ml in 10^{-4} M MgAc, pH 5.0; temperature, 1°C; pulse duration 1.0 msec. each polarity; field strength 6 kv/cm; wave length, 254 mμ; 0.20 cm gap.

4. DISCUSSION

The above data show that the ultraviolet-absorbing groups of ribosomes can be oriented in electric fields so that their transition moments are predominantly parallel to the field. The major members of these groups are the bases of the ribosomal ribonucleic acid. Their transition moments for 254 $m\mu$ absorption lie in their planes. Further, the observed birefringence implies that ribosomes have an electric axis which becomes oriented parallel to the applied electric field. Since the bases are also the most polarizable groups within ribosomes (again, in their plane) the direction of their greater refractive index lies parallel to the ribosomal electric axis and therefore the birefringence is positive.

It is possible that the ribosomal electric axis is also an axis of cylindrical or helical symmetry. A possible way the ribosomal RNA might be arranged is in segments of double-helices (in the manner of the Watson-Crick model of DNA) whose axes must then be prependicular to the main ribosomal axis. Such details are, however, unobtainable with the present technique. Nor can these results be simply related to the $\frac{1}{3}$-$\frac{2}{3}$ pattern of ribosomal dissociation, although it is quite possible that the electric axis is normal to the plane of this dissociation. The only way that the symmetry of the ribosome can be learned is by x-ray diffraction of oriented specimens. Whether electric orientation (which so far lasts only a millisecond) can be usefully coupled to x-ray diffraction (which conventionally takes exposures of many hours) remains an intriguing possibility for future exploration.

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Note Added in Proof Golub, Guse, Dvorkin, and Spirin (*Doklady,* 1963, **149,** 446) have recently observed positive electric birefringence from solutions of 100S and 70S ribosomes isolated from *E. coli* and treated with 4 per cent formaldehyde. These authors do not, however, interpret this effect in terms of the orientation of the ribosomal nucleic acid.