Conformation of Deoxyribonucleic Acid in Alcohol Solutions†

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ABSTRACT: The circular dichroism of a number of DNAs has been studied as a function of alcohol concentration for both methanol–buffer and ethanol–buffer solvent systems. In the range of 0–65% v/v ethanol and all ranges of methanol, the CD changes reflect a B- to C-form geometry transition of the type seen in dehydrated Li–DNA films, ethylene glycol, and high-salt solutions. Above an ethanol concentration of 65% DNA behaves in an anomalous fashion as monitored by a large change in the CD spectrum. An increase in scattering indicates that the DNA has condensed into a compact tertiary structure. T2 phage DNA, which has a CD spectrum characteristic of the C form in buffer, is found to undergo the same transition. In 80% ethanol this DNA melts with two transitions which we interpret first as a melting of the tertiary structure (T_m = 45°) and a return to the C form, and second as a melting of the C-form secondary structure (T_m = 54°). These results indicate that DNA behaves the same way in the two closely related solvent systems as long as the polymer is actually dissolved. The anomalous CD spectrum at high concentrations of ethanol results from changes associated with condensation or aggregation.

The conformation of DNA in solution is a function of temperature, pH, salt concentration, and solvent. In particular DNA shows remarkable behavior when dissolved in alcohols and its properties in ethanol and methanol have been studied extensively by a variety of means. Of particular interest is the circular dichroism (CD) work of Brahms and Mommaerts (1964). They reported a significant increase in magnitude of the first band of the CD spectrum of calf thymus DNA in 80% v/v ethanol–water when compared to that of DNA in buffer. Thus while DNA assumes the well-known B form in buffer, the CD spectrum in 80% ethanol resembles what one might expect for A-form DNA. Films of DNA in the A form have a similar large first CD band (Tunis-Schneider and Maestre, 1970) as does double-stranded RNA (Samejima *et al.*, 1968; Gratzer and Richards, 1971). This behavior is also predicted by theory (Johnson and Tinoco, 1969).

The authors became interested in the conformation and resulting optical properties of DNA in alcohols when we discovered independently that DNA assumes the C form in 95% v/v methanol–buffer solutions. Tunis-Schneider and Maestre (1970) had previously shown what CD spectrum one should expect for C-form DNA using films. This form of DNA has been found in ethylene glycol solutions (Nelson and Johnson, 1970; Green and Mahler, 1971) and high-salt solutions (Maestre and Tinoco, 1967; Tunis and Hearst, 1968; Tunis-Schneider and Maestre, 1970). It seems quite peculiar that DNA should assume the C-form conformation in ethylene glycol and 95% methanol, but yet assume the A-form conformation in the very similar 80% ethanol solvent system.

After this manuscript was submitted for publication, a CD study of DNA in alcohol–water solutions appeared (Ivanov *et al.*, 1973). Our results for the methanol–water system agree with theirs, but our results for the ethanol–water system differ. They studied DNA in ethanol–water only in the high ethanol concentration range and conclude that it goes directly from the B form to the A form. We consider this quite strange. Our treatment of this system is considerably more detailed and leads to a solution to this puzzle.

Materials. *Escherichia coli* DNA was extracted by the methods described by Thomas *et al.* (1966). Other *E. coli* DNA was purchased from Worthington Biochemical Corp. (DNAEC 90B). A stock solution of the DNA was prepared by dissolving it in the EDTA buffer (10^{-2} M NaCl–10^{-2} M Trisma base–10^{-3} M EDTA, pH 7.2). For measurements of CD and absorption, the stock solution was diluted tenfold with water and alcohol to give the desired alcohol content and a salt composition of 10^{-3} M NaCl, 10^{-3} M Trisma base, and 10^{-4} M EDTA.

Calf thymus DNA was purchased from Worthington Biochemical Corp. (DNACT 2DA). A stock solution of the DNA was prepared by dissolving it in standard sodium citrate buffer (SSC) × 10^{-1}. For measurements of CD and absorption the stock solution was diluted 20-fold with water and alcohol to give the desired alcohol content and a sodium ion concentration of 10^{-3} M.

T2 phage were grown and the DNA was extracted using methods described by Thomas and Abelson (1966). A stock solution of the phage DNA was prepared by dissolving it in 0.025 × SSC. This DNA was sheared by forcing it repeatedly through a no. 25 syringe needle. For optical measurements the solution was diluted fivefold with water and alcohol to give the desired alcohol content and a sodium ion concentration of 10^{-3} M.

All solution preparations were carried out in a cold room, and the stock solutions were diluted slowly while being stirred continuously. Solvent mixes were prepared on a volume basis. The DNA concentrations were kept low (0.15 OD/cm and below) and path lengths of 1 or 2 cm were used. The concentration of DNA in the solutions was calculated by assuming that the molar extinction coefficient for the native DNA in water is 6500 cm²/mol for *E. coli*, 6620 cm²/mol for calf thymus, and 6440 cm²/mol for T2, at the uv maximum of 258.7 nm.

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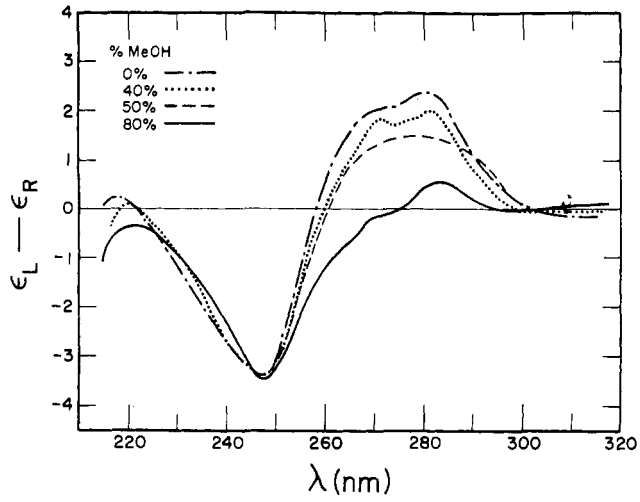

FIGURE 1: The CD spectra of *E. coli* DNA as a function of methanol concentration. Buffer: 10^{-3} M NaCl–10^{-3} M Tris–10^{-4} M EDTA. The CD at high methanol concentration shows C-type spectra.

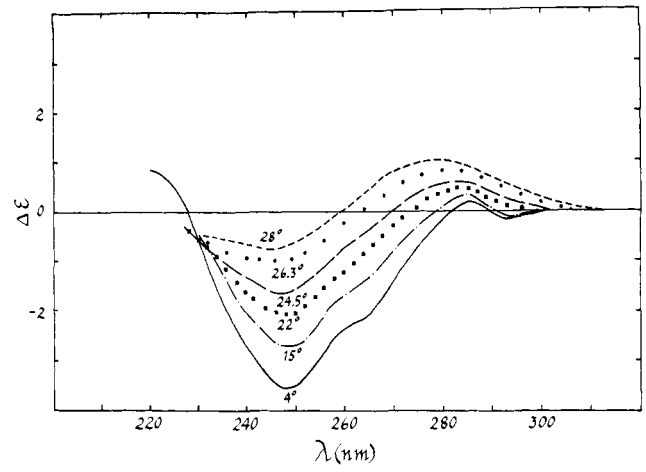

FIGURE 3: The CD spectra of calf thymus DNA in 95% methanol has a function of temperature. The 4° curve is typical of C-form DNA, and the 28° curve is typical of random coil.

Instrumentation. At Berkeley a Cary Model 14 spectrophotometer was used for measuring uv absorption and a Cary Model 6001 combined with a PDP8/S computer and an ASR teletype for CD measurements. Measurements were carried out at 25°. At Corvallis a Durrum-Jasco Model J-10 was used for both absorption and CD measurements. Most measurements were carried out at 8°.

The CD scattering correction measurements were made in a highly modified Cary 60 CD dichrograph at the Space Science Labs., Berkeley, Calif. The modification and the design of the integrating Fluorscat cell is described in Dorman and Maestre (1973).

Results and Discussion

The CD spectra of *E. coli* and calf thymus DNA in solutions of various methanol contents are given in Figures 1 and 2, respectively. In both cases the intensity of the 275-nm band decreases as the methanol content of the solution increases. At high percentages of methanol we obtain CD spectra which are characteristic of C-form DNA (Tunis-Schneider and Maestre, 1970). The results for calf thymus DNA in 95% methanol are nearly identical with the results for that type of DNA in ethylene glycol (Green and Mahler, 1971; Nelson and Johnson, 1970). Figure 3 shows the melting characteristics of calf thymus DNA in 95% methanol.

Figures 4, 5, and 6 present the CD spectra of *E. coli* and calf thymus DNA as a function of ethanol concentration. The CD spectra behave in the 0–70% ethanol concentration region in a manner quite similar to methanol (Figures 1 and 2). However, a most striking change in the CD spectra occurs in the 70–80% ethanol concentration regions for both DNAs. There is a sudden increase in the magnitude of the first peak (270 nm), a diminution in the values at the trough (243 nm), and an increase in the value at the second maximum (220 nm).

Changes in the conformation of DNA with increasing ethanol concentration are also evident in absorbance behavior (Brahms and Mommaerts, 1964). Figure 7 summarizes our results for *E. coli* DNA as a function of ethanol concentration. The DNA spectrum becomes hyperchromic and shifts in wavelength toward the red. At 80% ethanol there is an increase in absorbance values at 330 nm of about +0.05 OD unit/OD_{258} reflecting some measure of scattering. The corresponding 15% increase in absorbance at 260 nm can be completely accounted for by light scattering alone, assuming a $1/\lambda^4$ Rayleigh law type scattering correction. Increased

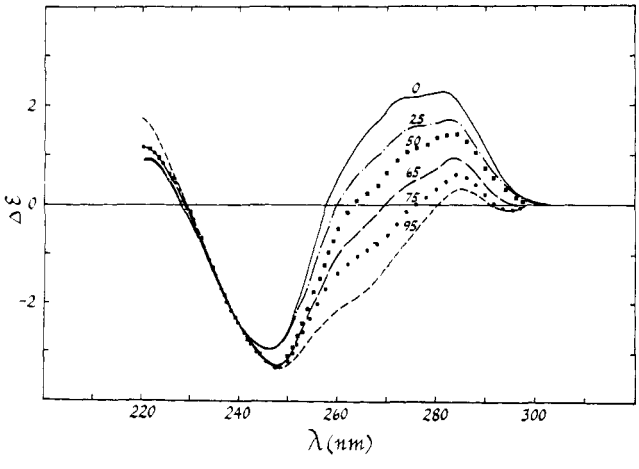

FIGURE 2: The CD spectra of calf thymus DNA as a function of methanol concentration. Buffer: 0.005 × SSC.

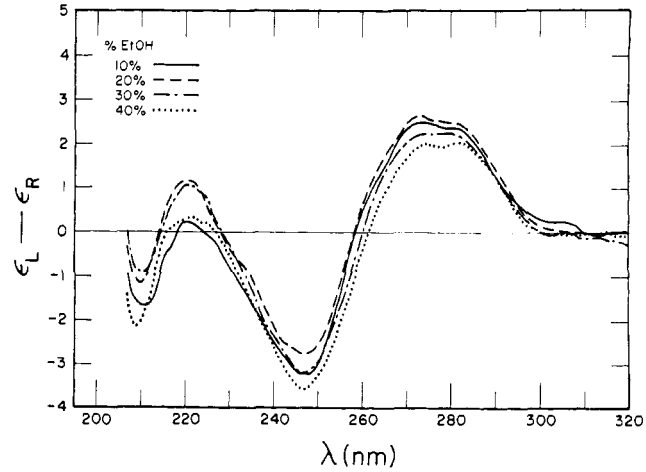

FIGURE 4: CD spectra of *E. coli* DNA *vs.* concentration of ethanol. Buffer: 10^{-3} M NaCl–10^{-3} M Tris–10^{-4} M EDTA. In this range there is a slight reduction of the maximum at 274 nm and a slight increase in the negative magnitude at the 243-nm minimum.

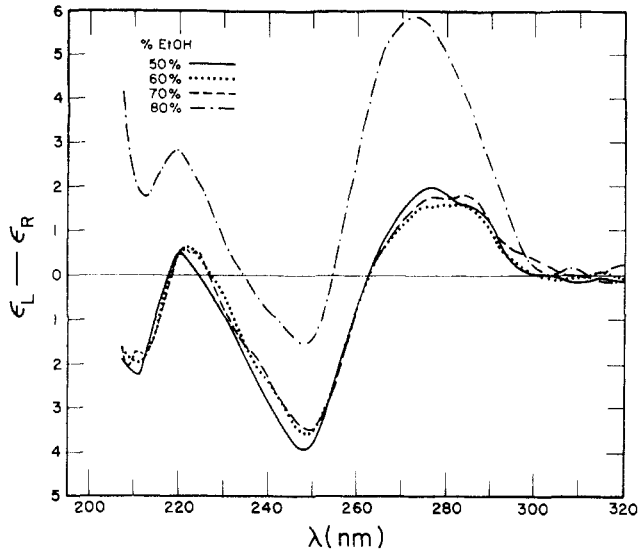

FIGURE 5: CD spectra of *E. coli* DNA *vs.* concentration of ethanol (50–80% v/v range). There is little change in spectra until the concentration of ethanol is in the range of 70–80%, where a radical alteration of the CD spectra occurs.

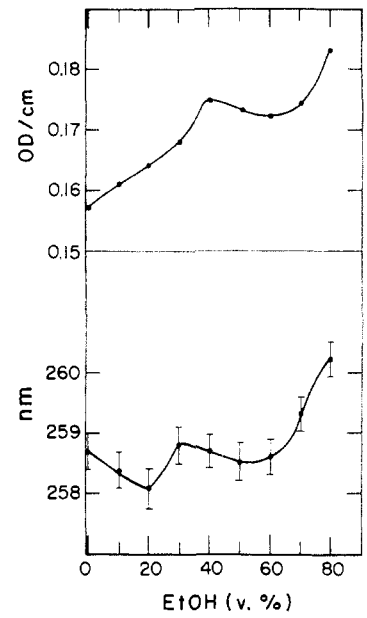

FIGURE 7: Upper graph: The optical absorbance of *E. coli* DNA as a function of percent ethanol. Buffer: 10^{-3} NaCl–10^{-3} M Tris–10^{-4} EDTA. Lower graph: positions of maxima of absorbance peak as a function of ethanol concentration.

scattering indicates the possibility of condensation of the DNA or aggregation at very high ethanol concentrations.

Sedimentation properties of DNA in ethanol led Geiduschek and Gray (1956) to conclude that DNA is in a collapsed state in this system. Coates and Jordan (1960) reached this same conclusion from further sedimentation experiments as did Herskovits *et al.* (1961) from viscosity, sedimentation, and light-scattering measurements. Such condensations of DNA have been reported by Dore *et al.* (1972) under the proper pH in ionic strength conditions. Lerman (1971) and Jordan *et al.* (1972) have found that DNA is driven into a compact state in solutions containing sufficient concentrations of simple neutral polymers and salts. Electron micrographs of a collapsed form of DNA precipitated from dilute ethanol solutions have been reported by Lang (1969).

The data presented indicate that the behavior of *E. coli* and calf thymus DNA in ethanol is probably no different from that of DNA in other dehydrating agents such as methanol, ethylene glycol, etc. This is apparently so until ethanol concentrations reach about 70% at which point there are sharp discontinuities in the optical behavior. The problem is whether the rapid changes measured in ethanol are due to intrinsic changes in the secondary structure of the DNA molecule as indicated by the A-form-like CD spectrum; or are the reflection of aggregation or condensation of the molecule just prior to precipitation from solution, as indicated by the light scattering.

It is dangerous to conclude that DNA in 80% ethanol is in the A form on the basis of CD measurements alone. Scattering

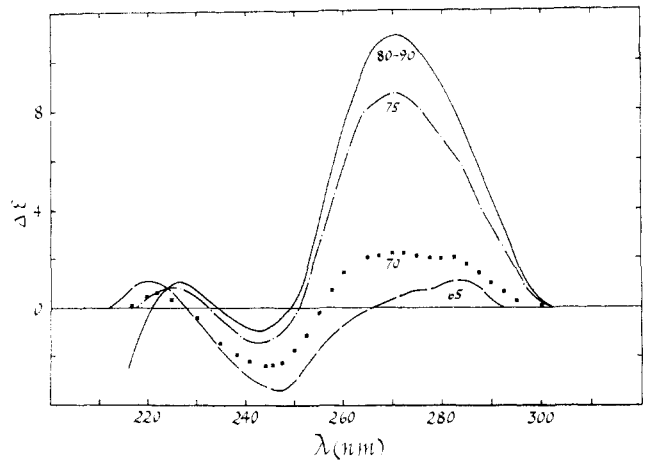

FIGURE 6: The CD spectra of calf thymus DNA as a function of ethanol concentration. Buffer: 0.005 × SSC. To 65% ethanol the spectra are identical with the methanol spectra presented in Figure 2.

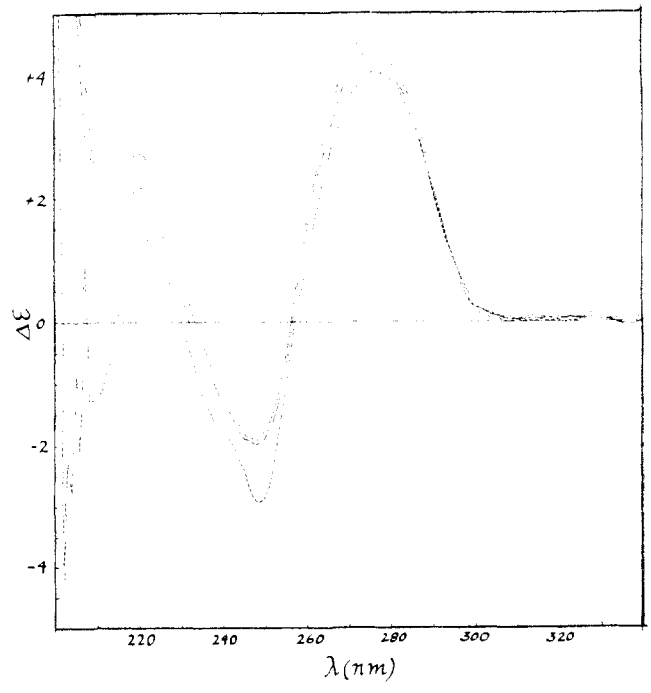

FIGURE 8: The CD spectra of *E. coli* DNA with various amounts of scattered light collected. The far position (—) has a half-angle of acceptance of about 10° and the near position (— — —) about 67°. The Fluorscat cell (- - - - - -) captures more scattered light than the near position.

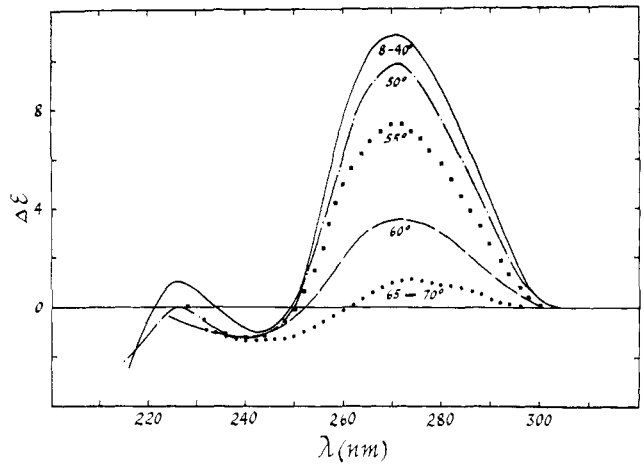

FIGURE 9: The CD of calf thymus DNA in 80% ethanol as a function of temperature.

from aggregated DNA could introduce artifacts which mimic the A-form spectrum and mask the true spectrum. Such alterations of CD spectra of aggregates can be seen in the work of Schneider *et al.* (1970) and in the studies on differential scattering of bacteriophage (Dorman and Maestre, 1972; Dorman *et al.*, 1973).

We measured the CD of the *E. coli* DNA in 80% ethanol as a function of scattered light collected (Figure 8) using a Fluoroscat (Dorman and Maestre, 1973). This indicates that a portion of the CD is due to light scattering. Unfortunately, the amount of correction given by the Fluorscat is unknown since we have no known calibrated differential scatterers. We hesitate to interpret the CD spectrum of DNA in 80% ethanol until the spectrum is completely corrected for scattering artifacts.

If the DNA is forming a compact tertiary structure in 80% ethanol as indicated by the light scattering, it might be possible to watch the tertiary structure melt out before the DNA denatures to a random coil. No such behavior is obvious for either the calf thymus (Figure 9) or the *E. coli* (similar, not shown) DNA in 80% ethanol. However, T2 phage DNA is found to melt with two transitions (Figure 10). This DNA exhibits the intense 250-nm band and weak 280-nm band in both buffer and 80% methanol which is characteristic of the CD spectrum of DNA in or approaching the C form (Figure 11). Although 70% glucosylated, it shows the same large 275-nm band in 80% ethanol as the nonglucosylated DNAs.

In 80% ethanol T2 DNA begins to melt about 35° as monitored by the 275-nm band (Figure 10). At 49° the 275-nm band has lost much of its intensity, but the 250-nm band has actually increased in intensity. The CD spectrum of T2 phage DNA at 49° is similar to the spectrum of that DNA in water and 80% methanol (Figure 11). Above 49° the magnitude of the 250-nm band decreases rapidly as the DNA denatures into a random coil whose CD spectrum is obtained at 60°. We believe that these melting curves show the tertiary structure formed by a T2 phage DNA to melt between 35 and 49° and the secondary structure to melt between 49 and 60°.

This spectrum provides a solution to the original puzzle. Why should DNA assume the C form in ethylene glycol and 95% methanol, but yet assume the A form in the very similar 80% ethanol solvent system? Apparently whenever DNA is truly dissolved it does behave the same way in these similar systems. The anomalous behavior results from aggregation or condensation of the DNA.

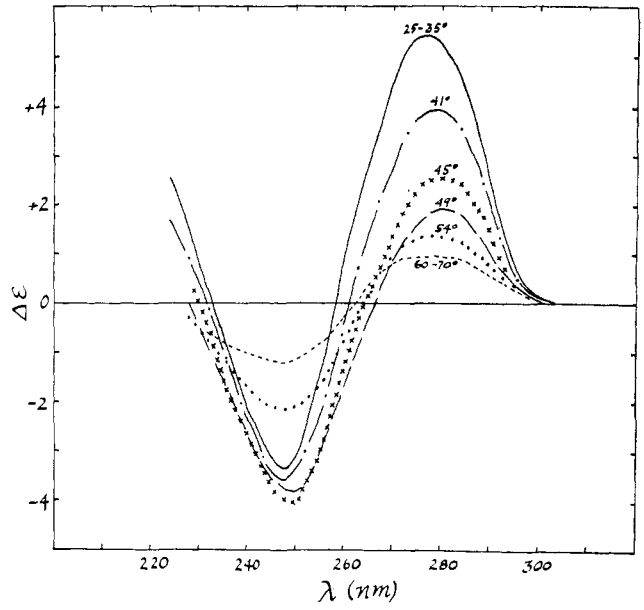

FIGURE 10: The CD of T2 phage DNA in 80% ethanol as a function of temperature. The 275-nm band melts between 35 and 49° while the 250-nm band melts between 49 and 60°.

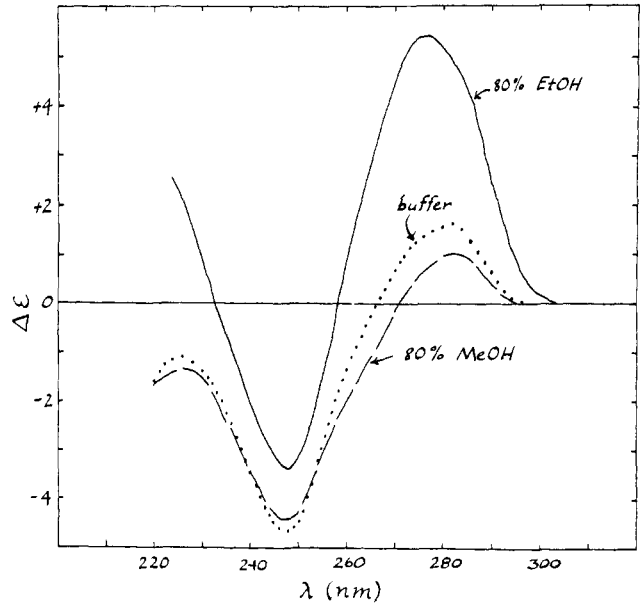

FIGURE 11: The CD of T2 phage DNA in buffer, 80% methanol, and 80% ethanol at 25°.

Acknowledgments

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Purification and Properties of Ribonucleic Acid Polymerase from Rat Liver Mitochondria†

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ABSTRACT: A DNA-dependent RNA polymerase was solubilized from mitochondrial membranes of rat liver and purified by differential and sucrose density gradient centrifugation and column chromatography on DEAE-Sephadex. The possibility of nuclear enzyme contamination of the mitochondrial enzyme preparation has been ruled out. The enzyme shows the requirement of Mn^{2+} and the presence of all four ribonucleoside triphosphates. It is completely insensitive to α-amanitin and is inhibited by rifampicin by 80% at 10 μg/ml. The enzyme activity is 80–90% dependent on added DNA template and both native calf-thymus DNA and mitochondrial DNA can serve as templates. The enzyme sediments at 4.3 S and appears to consist of one polypeptide chain with mol wt measured to be 66,000 on gel electrophoresis in the presence of sodium dodecyl sulfate.

Recent observations suggest that mitochondria possess a considerable degree of autonomy in their biogenesis (Slater *et al.*, 1968; Roodyn and Wilkie, 1968). It has been demonstrated in several organisms that mitochondria can also synthesize their own RNA (Wintersberger, 1964; Luck and Reich, 1964; Neubert and Helge, 1965; Kalf, 1964; Suyama and Eyer, 1968). Tsai *et al.* (1971) have solubilized and purified mitochondrial RNA polymerase from yeast and reported that it differs from the corresponding nuclear enzyme in lacking inhibition by α-amanitin.

RNA polymerase activity in rat liver mitochondria, either in the intact organelle or in the solubilized form, has also been reported by several investigators (Saccone and Gadaleta, 1970; Wintersberger and Wintersberger, 1970; Gadaleta *et al.*, 1970; Shmerling, 1969). Shmerling (1969) and Gadaleta *et al.* (1970) found that RNA polymerase activity in intact rat liver mitochondria was highly sensitive to rifampicin, whereas nuclear RNA polymerase activity was not.

Recently, Reid and Parsons (1971) reported the solubilization and partial purification of RNA polymerase from rat liver mitochondria. But these investigators found that the partially purified enzyme was only slightly inhibitable by rifampicin. This paper describes the isolation of membrane bound mitochondrial RNA polymerase from rat liver, its solubilization, and purification. This purified hepatic mitochondrial RNA polymerase was found to be highly sensitive to inhibition by rifampicin.

Materials

Rifampicin, a rifamycin derivative used for inhibition studies, was obtained from Calbiochem. α-Amanitin was a gift from Professor T. Wieland, Max-Planck Institute for Medical Research, Heidelberg, West Germany. All other biochemicals were obtained commercially.

† From the Cancer and Radiobiological Research Laboratory, Department of Health and Hospitals, Francis Delafield Hospital, New York, New York 10032, and the Department of Biology, New York University, New York, New York 10032. *Received February 21, 1973.* A preliminary account of parts of this work has appeared (Mukerjee and Goldfeder, 1972). This work was supported in part by grants from the National Institutes of Health and the Mildred Werner League for Cancer Research.

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