Keith, J., and Fraenkel-Conrat, H. (1975), *FEBS Lett. 57,* 31.
Moyer, S., Abraham, G., Adler, R., and Banerjee, A. K. (1975), *Cell 5,* 59.
Munns, T., Podratz, K., and Katzman, P. (1974), *Biochemistry 13,* 4409.
Perry, R. P., and Kelley, D. E. (1976), *Cell* (in press).
Perry, R. P., Kelley, D. E., Friderici, K. H., and Rottman, F. M. (1975a), *Cell 4,* 387.
Perry, R. P., Kelley, D. E., Friderici, K. H., and Rottman, F. M. (1975b), *Cell 6,* 13.
Perry, R. P., and Scherrer, K. (1975), *FEBS Lett. 57,* 73.
Rottman, F., Shatkin, A., and Perry R. (1974), *Cell 3,* 197.
Salditt-Georgieff, M., Jelinek, W., Darnell, J. E., Furuichi, Y., Morgan, M., and Shatkin, A. (1976), *Cell 7,* 227.
Sripati, C. E., Groner, Y., and Warner, J. R. (1976), *J. Biol. Chem. 251,* 2898.
Stuart, S. E., and Rottman, F. M. (1973), *Biochem. Biophys. Res. Commun. 55,* 1001.
Tener, G. M. (1967), *Methods Enzymol. 12,* 398.
Wei, C. M., Gershowitz, A., and Moss, B. (1975), *Cell 4,* 379.
Wei, C. M., and Moss, B. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 318.

Double-Stranded DNA in Methanol–Ethanol–Buffer Solvent System†

Ernest Kay

ABSTRACT: DNA in a solvent system consisting of roughly equal volumes of methanol and ethanol and 5% buffer has a conservative circular dichroism (CD) spectrum of very low intensity above 220 nm and an increase of ϵ_{258} comparable to that of denatured DNA (about 40%). A direct comparison of this spectrum with the CD of single-stranded DNA reveals many differences, indicating DNA in this solvent system has a conformation different from that of denatured DNA. When the alcohols are removed, the B form conformation and normal ϵ_{258} are restored in native DNA, while single-stranded DNA remains denatured. A double-stranded structure of DNA in the methanol–ethanol–buffer solvent system is confirmed by the neutral cesium chloride density gradient centrifugation of DNA in which one chain is labeled with [^{14}C]thymidine and the other [^{3}H]5-bromodeoxyuridine. The doubly labeled DNA exposed to the alcohol solvent system has a centrifugal pattern identical with that of control DNA; the two radioactivities cosediment and form a superimposing band, distinctly different from that of single-stranded DNA; ^{3}H-labeled (thymidine) chains sediment further than ^{14}C-labeled chains (5-bromodeoxyuridine). Denatured DNA exhibits varying CD spectra depending on solvents. It is suggested that single-stranded DNA in different solvent systems assumes different modes of base stacking.

Within the restriction of the Watson–Crick pairing scheme, complementary nucleic acids display a remarkable diversity of secondary structure. A variety of distinct conformations has been characterized in DNA fibers by the x-ray diffraction technique (Arnott, 1970). In solution, changes in conformation can be brought about by changes in pH, solvent, temperature, relative humidity, or nature and concentration of counterions, or by complexing with other macromolecules (Bush and Brahms, 1973). Circular dichroism (CD[1]) has been employed extensively to monitor these transformations as it is a very sensitive technique to measure conformational changes in polynucleotides. Such studies may permit an assessment of the relative importance of different conformational forces in conferring the stability of DNA in solution.

Conformational changes of DNA in different solvents have attracted considerable interest. Mixed-type media are commonly used with water as one of the components. Low-molecular-weight alcohols are often used as the second component because of their low polarity and ability to mix well with water. For example, DNA in buffer assumed the B form but transforms to the C form in the 95% methanol–5% buffer and 65% ethanol–35% buffer solvent systems (Girod et al., 1973). In contrast to these organic solvent systems where DNA is found to retain a secondary structure, it was reported to be completely and irreversibly denatured and lose all secondary structure in a solvent system which contained roughly equal volumes of methanol and ethanol and a buffer concentration below 15% (Johnson and Girod, 1974). This conclusion was based on ultraviolet absorption and CD studies. No cogent interpretation was given to this unexpected finding.

However, a close examination of the reported CD spectra (Johnson and Girod, 1974) revealed that the intensity of two CD bands above 230 nm was too low compared with that of denatured DNA, even compared with that of spectra recorded at the denaturing temperature (Brahms and Mommaerts, 1964; Usatyi and Shlyakhtenko, 1973). Moreover, the validity of comparing absorption and CD spectra in buffer and those in alcohols has never been established. Because of these uncertainties, the structure of DNA in the methanol–ethanol–buffer solvent system has been reexamined using the same spectroscopic techniques. The results show that native and heat-denatured DNA have different CD spectra in the meth-

† From the Laboratory of Nuclear Medicine and Radiation Biology, University of California, Los Angeles, California 90024. *Received May 3, 1976.* This study was supported by Contract E(04-1) GEN-12 between the Energy Research and Development Administration and the University of California, by the Division of Cancer Treatment Contract N01-CM-43791, and by the National Science Foundation Grant MBS 74-13253 A02.

[1] Abbreviations used: CD, circular dichroism; SSC, standard saline-citrate buffer (150 mM NaCl, 15 mM Na citrate, pH 7.5); dThd, thymidine; BrdUrd, 5-bromodeoxyuridine; $\Delta\epsilon$, molar extinction coefficient for left circularly polarized light minus that for right circularly polarized light; EDTA, ethylenediaminetetraacetic acid.

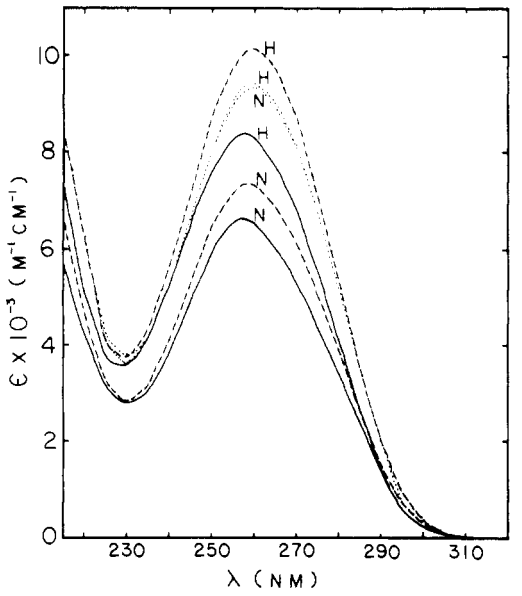

FIGURE 1: Absorption spectra of calf thymus DNA. N, native DNA; H, heat-denatured DNA. Solvents are: (—) SSC × 10^{-1}; (---) 95% methanol–5% SSC × 10^{-1}; (. . .) 50% methanol–45% ethanol–5% SSC × 10^{-1}. Heat-denatured DNA was prepared by heating DNA solution in boiling water for 10 min and then chilled rapidly in ice.

anol–ethanol–buffer solvent systems. Upon removal of alcohols, the former restored B form, the native CD spectrum in buffer, while the latter remained denatured. Results of neutral CsCl density gradient centrifugation study indicate that native DNA remains double-stranded in this solvent system. Presented for comparison are data on DNA treated with 95% methanol–5% buffer in which DNA has been known to maintain double-stranded structure (Girod et al., 1973).

Experimental Procedure

Materials. Calf thymus DNA (Calbiochem, A grade) was treated with boiled RNase at 37 °C for 30 min, then deproteinized with freshly distilled phenol, precipitated with chilled ethanol, redissolved in 10 mM $NaClO_4$, and dialyzed exhaustively against cold SSC × 10^{-1}. *Escherichia coli* DNA was prepared from *E. coli* $15T^-$ culture. Cells at the late log phase were harvested and washed with 0.15 M NaCl–0.1 M EDTA, pH 8.0. Cell lysis and protein digestion were accomplished by Pronase in 1% sodium dodecyl sulfate–SSC at 37 °C for 7 h. Residual protein was removed by phenol extraction and DNA precipitated with 2 volumes of ice-cold ethanol. After redissolving in SSC × 10^{-1}, RNA was removed by RNase treatment and DNA precipitated with 0.54 volume of 2-propanol. DNA was redissolved in 10 mM $NaClO_4$ and dialyzed against SSC × 10^{-1}. Heat-denatured DNA was prepared by heating DNA–SSC × 10^{-1} in boiling water for 10 min and then was chilled rapidly in ice. DNA in the final preparations was sheared by forcing through a 27-gauge needle five times at 0 °C, and then was diluted 20-fold with SSC × 10^{-1} or alcohol. For the 50% methanol–45% ethanol–5% buffer solvent system, DNA in SSC × 10^{-1} was mixed thoroughly with methanol before adding ethanol. Differential changes of DNA concentration in alcohol-treated samples during dialysis vs. SSC × 10^{-1} were equalized by adjusting sample volume with SSC × 10^{-1} before absorption and CD measurements.

Spectrophotometric Analyses. Absorption spectra were recorded on a Cary Model 15 spectrophotometer. CD spectra were measured with an instrument described elsewhere (Horwitz et al., 1968; Horwitz, 1970; Fretto, 1972). CD spectra presented are derived from the average of four scans, except for those at 90 °C in which only two scans were measured. For spectra recorded at 90 °C, cuvette temperature was regulated by a Beckman T_m analyzer with a platinum probe. A time span of 10 min was used to raise the temperature from 24 to 90 °C. Other CD spectra and absorption spectra were recorded at 24 °C. DNA concentration in buffer was estimated using values of ϵ_{260} 6600 (Mahler et al., 1964) and M_r = 340 per nucleotide.

Neutral Cesium Chloride Density Gradient Centrifugation. Doubly labeled DNA was prepared by the late Dr. Y. C. Lee from murine leukemia L1210 cells. Roswell Park Memorial Institute medium 1630 (Associated Biomedic Systems) supplemented with 6% fetal calf serum was used for culture. Cells were grown in the medium containing [^{14}C]dThd (0.25 μCi/ml) for one generation (16 h) and then transferred to a fresh medium in which [^{3}H]BrdUrd (5 μg/ml, 0.5 μCi/ml) substituted for the thymidine. After one generation (23 h) in the BrdUrd medium, DNA was extracted according to a modified Marmur procedure (Byfield et al., 1972) and dialyzed against SSC × 10^{-1}. DNA was sheared by forcing through a 27-gauge needle five times at 0 °C, treated with an appropriate solvent system, held at 24 °C for 2 h, and dialyzed against cold SSC × 10^{-1} to remove alcohols. For centrifugation 5.625 g of CsCl (Harshow, optical grade) was dissolved in 3.8 ml of DNA solution and centrifuged in a fixed angle Beckman 40.2 rotor at 38 000 rpm for 37 h at 10 °C. For an analysis in the presence of alcohol, a 0.2-ml aliquot was removed from alcohol-treated samples before dialysis and diluted with 3.6 ml of SSC × 10^{-1} before dissolving CsCl. Following centrifugation, the bottom of polyallomer tubes was punctured with a needle and fractions were collected on filter paper discs (Whatman No. 3 MM, 2.3 cm). The discs were air-dried and assayed for ^{3}H and ^{14}C in an Isocap/300 liquid scintillation system (Nuclear Chicago). The total radioactivity per tube of DNA with alcohol was approximately one-tenth of DNA with alcohol removed. Efficiency of ^{3}H and ^{14}C channels and crossing-over of ^{14}C channel to ^{3}H channel were determined by counting [^{3}H]DNA or [^{14}C]DNA of known activity (mixed in 0.5 ml of SSC × 10^{-1} 60% CsCl) on the filter paper discs.

Other reagents used were of analytical grade. Double-distilled water was used throughout.

Results

The absorption spectra of native and heat-denatured DNA from calf thymus and *E. coli* in SSC × 10^{-1}, 95% methanol–5% SSC × 10^{-1}, and 50% methanol–45% ethanol–5% SSC × 10^{-1} are presented in Figures 1 and 2. In both calf thymus and *E. coli*, denatured DNA is less hypochromic compared with the native control in the same solvent. A decrease in the hypochromicity was also exhibited by both native and denatured samples by changing solvents from buffer to alcohol–buffer solvent systems, with methanol–ethanol–buffer least hypochromic. The difference of ϵ_{258} between the aqueous buffer and the ethanol–buffer solvent system in native DNA was about 40% (41.3% for calf thymus and 38.5% for *E. coli* DNA), a value in close agreement with a reported observation (Johnson and Girod, 1974).

CD spectra of DNA in SSC × 10^{-1} are depicted in Figures 3 and 4. At 24 °C, DNA from both calf thymus and *E. coli* has a conservative CD spectrum above 230 nm with an intense positive band around 275 nm and a negative band of comparable intensity around 245 nm. This kind of CD spectrum has been identified with the B form of DNA conformation (Johnson and Tinoco, 1969; Wooley and Holzwarth, 1971).

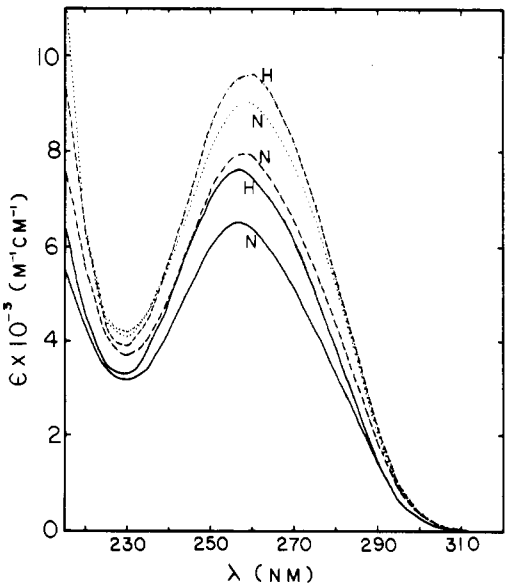

FIGURE 2: Absorption spectra of *E. coli* DNA. Symbols are the same as those in Figure 1.

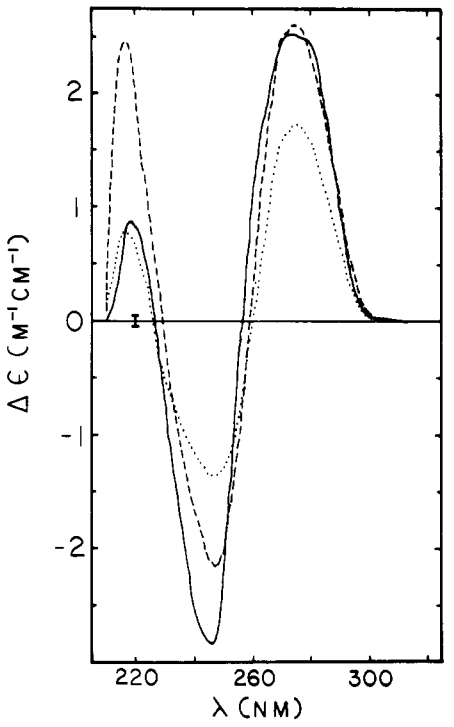

FIGURE 3: CD spectra of calf thymus DNA in SSC × 10^{-1}. (—) Native DNA at 24 °C; (---) heat-denatured DNA at 24 °C; (. . .) DNA at 90 °C. Bar on baseline indicates peak-to-peak noise level around 220 nm.

At 90 °C the intensity of both bands was much reduced, with a greater change in the 245-nm negative band. The conservative spectrum of reduced intensity is typical of DNA above melting temperature (Brahms and Mommaerts, 1964; Usatyi and Shlyakhtenko, 1973). The intensity of the 275- and 245-nm bands is increased when the heated DNA is chilled rapidly. The greatest difference is noted around 220 nm where a positive peak became much greater in heat-denatured samples recorded at 24 °C. CD of alkali-denatured calf thymus DNA (treated with 0.1 N NaOH, dialyzed against SSC × 10^{-1}) is identical with that of heat-denatured DNA at room temperature (Figure 3, dashed line).

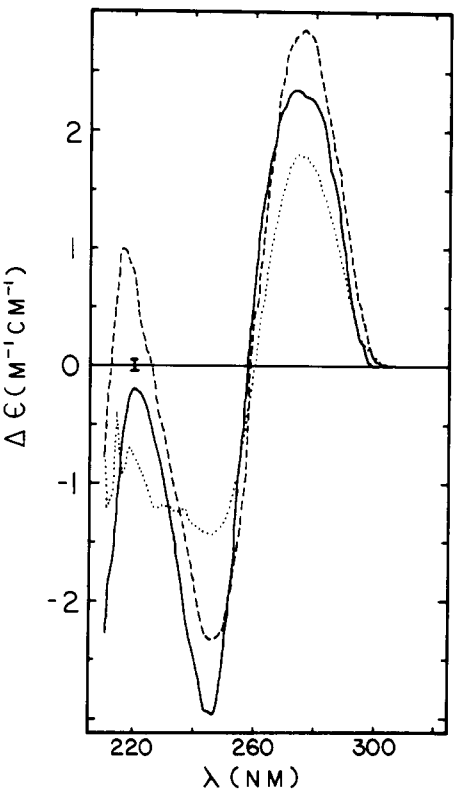

FIGURE 4: CD spectra of *E. coli* DNA in SSC × 10^{-1}. Symbols are the same as those in Figure 3.

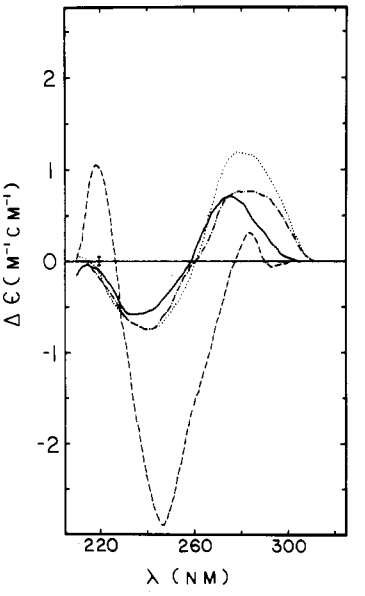

FIGURE 5: CD spectra of calf thumus DNA in alcohol solvents. (—) Native DNA in 50% methanol-45% ethanol-5% SSC × 10^{-1}; (---) native DNA in 95% methanol-5% SSC × 10^{-1}; (- · · · · ·) heat-denatured DNA in 50% methanol-45% ethanol-5% SSC × 10^{-1}; (. . .) heat- denatured DNA in 95% methanol-5% SSC × 10^{-1}.

Figures 5 and 6 show CD of DNA in alcohol solvent systems. In 95% methanol-5% SSC × 10^{-1}, native DNA of both calf thymus and *E. coli* has a CD spectrum suggested for C form (Tunis-Schneider and Maestre, 1970; Girod et al., 1973). Heat-denatured samples in this solvent system have a conservative CD of much reduced intensity (a negative shoulder in *E. coli*), which is clearly different from DNA in SSC × 10^{-1} at 90 °C or heat-denatured DNA at 24 °C (Figures 3 and 4).

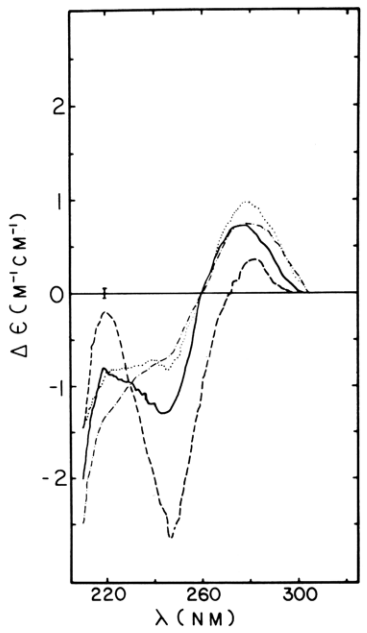

FIGURE 6: CD spectra of *E. coli* DNA in alcohol solvents. Symbols are the same as those in Figure 5.

In 50% methanol–45% ethanol–5% buffer, CD spectra of native and heat-denatured DNA are somewhat similar and are of conservative and very low intensity. However, nonidentity of the spectra between native and heat-denatured samples is readily apparent. They are also distinctly different from those of denatured DNA in buffer alone. The profile of native calf thymus DNA in this solvent system (Figure 5, solid line) is essentially the same as that recorded under a similar condition in the earlier report (Johnson and Girod, 1974).

When alcohols were removed from native calf thymus DNA by dialyzing against SSC × 10^{-1}, ϵ_{258} returned to that of native DNA in buffer (6600) and the B form CD spectrum, identical with control native DNA (exposed only to SSC × 10^{-1}), was regained (Figure 7, N). Heat-denatured calf thymus DNA treated similarly had CD spectrum indistinguishable from that of heat-denatured DNA in SSC × 10^{-1} (Figure 7, H).

Sedimentation patterns of doubly labeled DNA in the neutral CsCl density gradient centrifugation are shown in Figure 8. In double-stranded control sample (Figure 8A), [^{14}C]dThd-labeled chains and [^{3}H]BrdUrd chains cosedimented near the top of the gradient. An over-labeling (more than one generation) of [^{3}H]BrdUrd is indicated by a heavier band labeled with only [^{3}H]BrdUrd (both chains labeled with [^{3}H]BrdUrd) at about half-way down the centrifugal tube. In the single-stranded control (Figure 8B), heavy chains labeled with [^{3}H]BrdUrd moved toward the bottom of the gradient, while light chains with [^{14}C]dThd remained at the top. A residual ^{3}H activity in this band is due to the over-labeling of [^{3}H]BrdUrd.

DNA exposed to either of the alcohol–buffer solvent systems and the alcohols removed by dialysis showed centrifugal patterns (Figure 8C and D) expected for double-stranded DNA (Figure 8A). These patterns were clearly different from those of heat-denatured DNA with single-stranded BrdUrd DNA toward the bottom of gradient and dThd chains near the top (Figure 8B).

DNA in the alcohol–buffer solvent systems was also centrifuged without dialysis and removal of alcohol. Centrifugal patterns of these samples were different from those of controls, perhaps due to the presence of a small amount of methanol (Figure 8E) and methanol–ethanol (Figure 8F). Double-stranded structure under these conditions, however, can be concluded from the superimposing radioactivities of [^{14}C]dThd

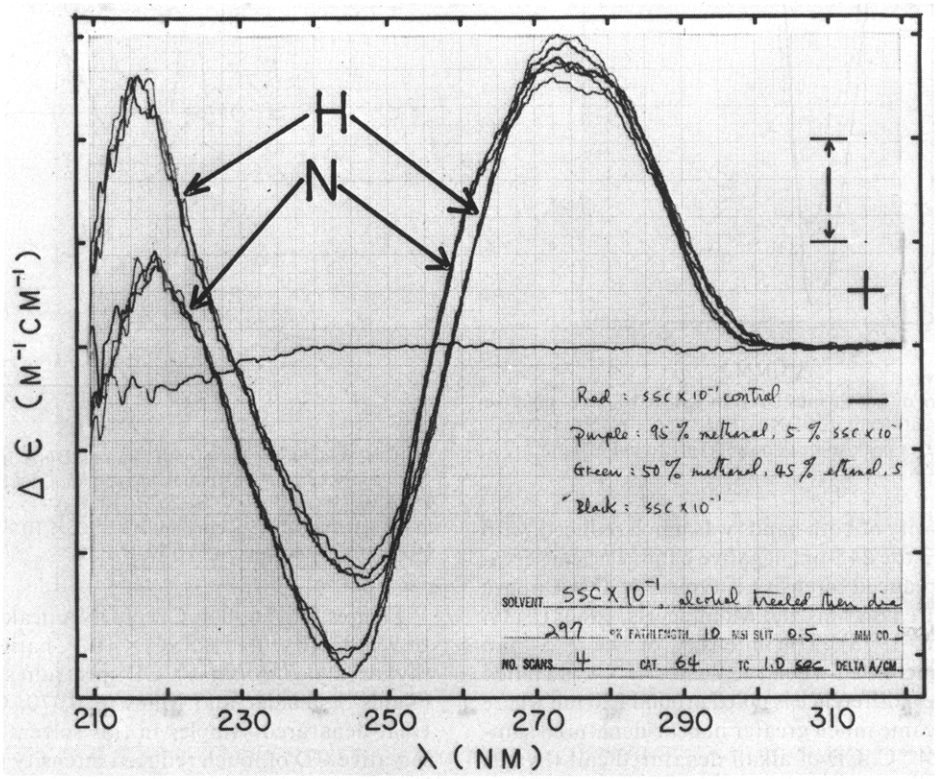

FIGURE 7: Instrument traces of native and heat-denatured calf thymus DNA exposed to alcohol solvents then alcohols removed by dialyzing against SSC × 10^{-1}. (N) Native DNA; (H) heat-denatured DNA. Each of three traces in both groups was exposed to either 50% methanol–45% ethanol–5% SSC × 10^{-1}, 95% methanol–5% SSC × 10^{-1}, or SSC × 10^{-1} (control) for 2 h at 24 °C and then dialyzed against cold SSC × 10^{-1}.

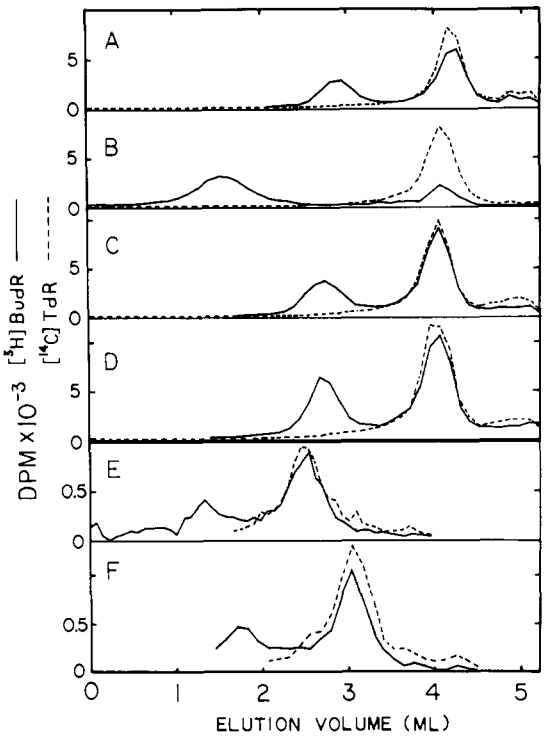

FIGURE 8: Centrifugal patterns of doubly labeled L1210 DNA in neutral CsCl density gradient. (A) Native DNA; (B) heat-denatured DNA; (C) native DNA exposed to 95% methanol-5% SSC × 10^{-1} and then alcohol removed by dialysis; (D) native DNA exposed to 50% methanol-45% ethanol-5% SSC × 10^{-1} and then alcohol removed by dialysis; (E) native DNA without removing 95% methanol-5% SSC × 10^{-1}; (F) native DNA without removing 50% methanol-45% ethanol-5% SSC × 10^{-1}.

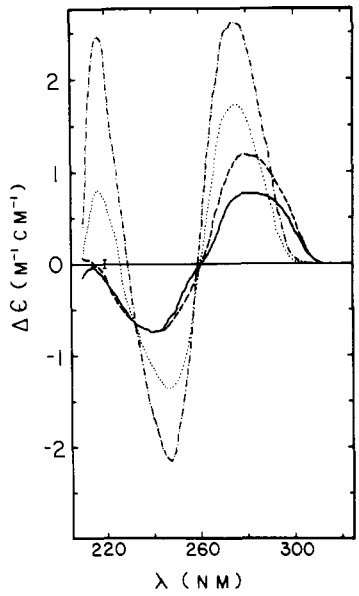

FIGURE 9: CD spectra of denatured calf thymus DNA in different solvents. (—) In 50% methanol-45% ethanol-5% SSC × 10^{-1}; (---) 95% methanol-5% SSC × 10^{-1}; (- - - - -) SSC × 10^{-1} at 24 °C; (. . .) SSC × 10^{-1} at 90 °C.

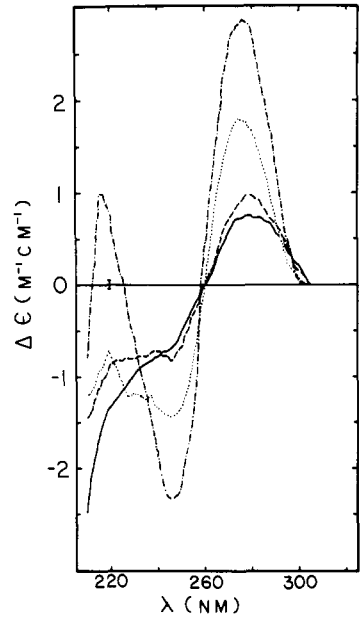

FIGURE 10: CD spectra of denatured *E. coli* DNA in different solvents. Symbols are the same as those in Figure 9.

and [^{3}H]BrdUrd in the major band.

Discussion

It is apparent from the comparison of CD spectra that DNA dissolved in the methanol-ethanol-buffer solvent system has a structure different from commonly identified DNA conformations (A, B, and C, Bush and Brahms, 1973; Ivanov et al., 1973) or that of denatured DNA (Brahms and Mommaerts, 1964; Usatyi and Shlyakhtenko, 1973). A double-stranded nature of this conformation is indicated by the restoration of the B form and ϵ_{258} of native DNA upon the removal of alcohols by dialysis against SSC × 10^{-1}. Reannealing of single-stranded DNA during the dialysis is not likely because heat-denatured DNA treated similarly remained single stranded. This conclusion is confirmed by the neutral CsCl gradient centrifugation study of doubly labeled DNA. Again a possibility of reannealing denatured DNA during dialysis can be ruled out because the alcohol-treated samples without dialysis also maintain a double-stranded structure.

A similar observation that DNA in solvent system consists of roughly equal volumes of methanol and ethanol and a buffer concentration below 15% has conservative CD of very low intensity reported earlier was interpreted differently (Johnson and Girod, 1974), mainly because these authors compared spectra obtained in the methanol-ethanol-buffer solvent system with that of denatured DNA in buffer alone. The present study has demonstrated an inadequacy in directly comparing the spectra measured in different solvent systems. An increase of about 40% in ϵ_{258} indicates the lack of hypochromism in DNA dissolved in the methanol-ethanol-buffer solvent system (Beaven et al., 1955). Lessened hypochromic effect in alcohol solvent systems relative to aqueous solvents is expected because of the lower polarity of alcohols (dielectric constants of water, methanol, and ethanol at 25 °C are: 78.54, 32.63, and 24.30, respectively, Suzuki, 1967). It should be pointed out that the lack of hypochromism does not necessarily mean the absence of interaction between neighboring bases, though its presence is the evidence of base interactions.

Single-stranded DNA in different solvent systems has different CD spectra (Figures 9 and 10). Apparently single-stranded DNA has ordered structures in solution and assumes different modes of base stacking when dissolved in different solvent systems.

Acknowledgment

I thank the late Dr. Y. C. Lee for preparing radioactive DNA, Ms. Irene Kuo for preparing *E. coli* DNA, and Dr. Joseph Horwitz for valuable discussions during the preparation of the manuscript.

References

Arnott, S. (1970), *Prog. Biophys. Mol. Biol. 21,* 265.
Beaven, G. H., Holiday, E. R., and Johnson, E. A. (1955), in The Nucleic Acids, Vol. 1, Chargaff, E., and Davidson, J. N., Ed., New York, N.Y., Academic Press, p 493.
Brahms, J., and Mommaerts, M. M. W. F. (1964), *J. Mol. Biol. 15,* 467.
Bush, C. A., and Brahms, J. (1973), in Physical Chemical Properties of Nucleic Acids, Vol. 2, Duchesne, J., Ed., New York, N.Y., Academic Press, p 147.
Byfield, J. E., Lee, Y. C., and Bennett, L. R. (1972), *Radiology 103,* 201.
Fretto, L. J. (1972), Ph.D. Thesis, University of California, Los Angeles, Calif.
Girod, J. C., Johnson, W. C., Jr., Huntington, S. K., and Maestre, M. F. (1973), *Biochemistry 12,* 5092.
Horwitz, J. H. (1970), Ph.D. Thesis, University of California, Los Angeles, Calif.
Horwitz, J., Strickland, E. H., and Kay, E. (1968), *Anal. Biochem. 23,* 363.
Ivanov, V. I., Minchenkova, L. E., Schyolkina, A. K., and Poletayev, A. I. (1973), *Biopolymers 12,* 89.
Johnson, W. C., Jr., and Girod, J. C. (1974), *Biochim. Biophys. Acta 353,* 193.
Johnson, W. C., Jr., and Tinoco, I., Jr. (1969), *Biopolymers 7,* 727.
Mahler, H. R., Kline, B., and Mehrotra, B. D. (1964), *J. Mol. Biol. 9,* 801.
Suzuki, H. (1967), Electronic Absorption Spectra and Geometry of Organic Molecules, New York, N.Y., Academic Press, p 93.
Tunis-Schneider, M., and Maestre, M. (1970), *J. Mol. Biol. 52,* 521.
Usatyi, A. F., and Shlyakhtenko, S. (1973), *Biopolymers 12,* 45.
Wooley, S. Y., and Holzwarth, G. (1971), *J. Am. Chem. Soc. 93,* 4066.

Conformational Changes of 30S Ribosomes Measured by Intrinsic and Extrinsic Fluorescence†

Pnina Spitnik-Elson, Nisson Schechter,‡ Renne Abramovitz and David Elson*

ABSTRACT: The intrinsic tryptophan fluorescence and the fluorescence of *N*-(3-pyrene)maleimide, a covalently bound sulfhydryl-specific extrinsic probe, have been used to study the conformation of the 30S ribosomal subunit of *Escherichia coli*. (a) The tryptophan fluorescence spectrum of the free ribosomal proteins is shifted to shorter wavelengths than that of free tryptophan. When the proteins are incorporated into the organized structure of the ribosome, there is a small additional blue shift and the emission band becomes narrower. In 6 M urea, the spectrum of the proteins, whether free or in the ribosome, becomes identical with that of the amino acid, reflecting exposure of previously shielded tryptophan residues. (b) When magnesium-depleted ribosomes are unfolded at low ionic strength, the tryptophan fluorescence spectrum changes, although circular dichroism shows no change in α-helix content of the proteins. (c) Intrinsic and extrinsic fluorescence were both found to be sensitive to a limited and fully reversible transition that takes place when ribosomes are incubated under conditions that increase their activity in vitro. This suggests that both probes may be of use in monitoring conformational changes that occur under conditions consistent with activity. The kinetics of the concurrent changes in extrinsic fluorescence and aminoacyl-tRNA binding activity were compared. (d) Conditions are described for labeling ribosomes with *N*-(3-pyrene)maleimide without impairing their activity.

The biological role of the ribosome calls for it to participate in a complex and repeated series of reactions and interactions, during the course of which the particle is believed to go through a cycle of conformational transitions. It is important to develop techniques that can detect such conformational changes, monitor them while they take place, and supply information on the nature of the change. Fluorescence spectroscopy, a sensitive and versatile technique for studying macromolecular conformation, should be useful in all of these respects. The technique has been applied to the ribosome in several different ways (Barenboim et al., 1969; Daya-Grosjean et al., 1972; Lemieux and Gerard, 1973; Hsiung and Cantor, 1973; Pochon and Ekert, 1973; Pochon et al., 1974; Huang and Cantor, 1975; Huang et al., 1975; Gerard et al., 1975; Schechter et al., 1975), but its use in this field is still at an early stage.

In the work described here, we have used two fluorescent probes as indicators of conformational change in the 30S ribosomal subunit of *E. coli:* the intrinsic tryptophan residues of the ribosome and an extrinsic covalently attached probe, *N*-(3-pyrene)maleimide, a specific sulfhydryl reagent that becomes fluorescent only after reacting covalently with a sulfhydryl group (Weltman et al., 1973). We have compared the tryptophan fluorescence of the ribosomal proteins free in

† From the Biochemistry Department, The Weizmann Institute of Science, Rehovot, Israel. *Received October 15, 1975.* Partly supported by Grant 394 from the United States-Israel Binational Science Foundation.

‡ Weizmann Fellow of the Weizmann Institute of Science. Current address: Department of Biochemistry, State University of New York, Stony Brook, N.Y. 11790.