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The circular dichroism and X-ray diffraction of DNA condensed from ethanolic solutions

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ABSTRACT

It is known that DNA in aqueous-ethanol solutions undergoes a B to A conformational change between 60% and 80% (w/w) ethanol. We have found that precipitates formed by adding salt to DNA in 60% and 80% ethanolic solutions can be very different. DNA precipitated from 60% ethanol forms a fine condensate that only slowly settles out of suspension and shows a characteristic differential scattering of circularly polarized light at long wavelengths. DNA precipitated from 80% ethanol forms a flocculent aggregate that exhibits the CD spectral features of the A conformation. Data from circular dichroism spectra of natural and synthetic nucleic acids and from X-ray diffraction patterns of the precipitates show that DNA molecules precipitated from 60% and 80% ethanol are, respectively, in the B and A conformation. Therefore, the different secondary conformations of DNA in ethanolic solutions are maintained during precipitation under these conditions. These results are of general importance for the preparation and study of condensed forms of DNA, since a relatively small change in the extent of dehydration can change the secondary conformation of DNA and markedly affect the character of a subsequent precipitate.

INTRODUCTION

Double-stranded DNA can exist in at least two distinctively different secondary conformations in fibers, as determined by X-ray diffraction. DNA in fibers containing about 6% NaCl exist in the B conformation at high (92%) relative humidity and in the A-conformation at low (75%) relative humidity.[1] The B conformation has 10 base pairs per 33.8 Å pitch; the base pairs are stacked along the helix with a tilt of -5.9° from the perpendicular.[2,3] The A conformation has 11 base pairs per 28.2 Å pitch; the base pairs are removed 4.7 Å from the helix axis and have a tilt of 20.2° from the perpendicular.[2] The deoxyribose sugars are in C'3-exo and C'3-endo conformations in the DNA B and A secondary conformations, respectively.[2,3] Double-stranded RNAs are restricted at all relative humidities to secondary conformations that are close to the DNA A conformation.[2,4]

Evidence from circular dichroism (CD) studies of solutions has shown

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that natural and synthetic DNAs, but not RNAs, will undergo a B to A conformational change upon dehydration by added ethanol.[5,6] At ethanol concentrations above 60% (w/w) the CD spectrum of DNA dramatically changes to become like that of RNA. The CD spectrum of RNA, on the other hand, is very little changed by the addition of ethanol.[6]

The fact that DNA precipitates in the presence of ethanol and sufficient salt is well-known and is commonly used as part of DNA isolation procedures.[7] In a study of the effect of ethanol on the CD spectrum of DNA, Girod et al.[8] concluded that the change to an RNA-like spectrum at 80% ethanol (1mM Na^+) coincided with the precipitation of the DNA. Other workers subsequently established the spectral change as being caused by a change in the secondary conformation of the DNA to the A conformation, without necessarily being accompanied by precipitation.[5,6] We now have studied the precipitation of DNA at various ethanol concentrations, by adding salt to DNA in aqueous ethanol solutions and following the CD spectral changes. We find that when DNA is precipitated at 80% ethanol, the RNA-like (A type) CD spectrum is maintained by the precipitating molecules, in agreement with the data of Girod et al.[8] By X-ray diffraction of such precipitates, we confirm that they contain DNA molecules in the A conformation. However, when DNA is precipitated at ethanol concentrations below those necessary to produce the B to A conformational change, it forms a much different type of precipitate, with unusual CD properties. By X-ray diffraction, we find that this latter precipitate contains molecules in the B secondary conformation.

Our results imply that the CD spectral identification of the DNA B and A conformations in 60% and 80% ethanolic solutions, respectively, is correct and that the secondary conformation of DNA is maintained during precipitation. We have also found that the type of precipitate formed from the B conformation has unique CD spectral contributions from the tertiary structure. These results provide new information for the design of experiments (e.g. for electron microscopy) that involve purification or preparation of DNA by precipitation from ethanolic solutions.

MATERIALS AND METHODS

Calf thymus DNA was purchased from Miles Laboratories and from Boehringer Mannheim. Miles DNA lot 3B, 0.3% protein by Lowry assay, was used for the CD spectra shown. Miles DNA batch 7018, 0.4% protein and 3.2% RNA, was used for combined X-ray diffraction and CD studies. Boehringer Mannheim DNA control no. 1367111, 1% RNA, molecular weight 10-12 x 10^6, was also used

for X-ray diffraction work. T2 DNA was phenol-extracted from bacteriophage purchased from Miles Laboratories (lot 6). Sonication of T2 DNA was performed for 2 min with a Branson Model S-75 low power sonicator and microtip. (We were not able to form a fine precipitate from 60% ethanol with intact T2 DNA of molecular weight 130 x 10^6.) Samples of synthetic poly[d(A-C)·d(G-T)] and poly[r(A-C)·r(G-U)], prepared as for previous work,[6] were generously provided by Dr. R. L. Ratliff (Univ. of California, Los Alamos Scientific Laboratory, New Mexico).

For the CD studies, all samples were dialyzed against a series of phosphate buffer solutions, first containing EDTA and then containing at least 0.01 M NaCl to aid in the removal of the EDTA. Finally, the samples were dialyzed against phosphate buffer (0.002 M Na^+, pH 7.0, except for poly-[d(A-C)·d(G-T)], which was at 0.001 M Na^+, pH 7.0). Absolute ethanol (U.S. Industrial Chemicals Company or Merck) was added to give the desired percent ethanol by weight. Salt concentrations in the DNA solutions were reduced by dilution as the ethanol was added. Then small aliquots of a concentrated NaCl solution were added without significant dilution of the ethanol, and the solutions were thoroughly mixed to form the precipitates.

For X-ray diffraction studies, two of the calf thymus DNA samples were prepared as follows: (1) The calf thymus DNA from Miles (batch 7018), which was supplied as a "salt-free" freeze-dried sample, was dissolved in water to give a 10% (w/w) gel; this was used to obtain the 0% ethanol pattern shown in Figure 8(a). A portion of this gel was diluted into 0.002 M Na^+ (phosphate buffer) for X-ray diffraction and parallel CD controls. (2) The calf thymus DNA from Boehringer Mannheim, which was supplied as a 0.3% DNA solution, was thoroughly dialyzed against 0.002 M Na^+ (phosphate buffer, pH 7.0). Ethanol was added to either of the DNA solutions to give 60% or 80% (w/w) ethanol. DNA concentrations were about 30 μg/ml and 14 μg/ml in 60% and 80% ethanol, respectively. Then salt was added to form precipitates.

For X-ray diffraction, the DNA was precipitated from 100 to 200 ml volumes by added salt. Especially for the precipitates from 60% ethanol, the DNA was allowed to settle slowly out of solution, for several hours or overnight. If necessary, the precipitate was drawn up into a pipette and allowed to settle and concentrate further. Concentrated precipitate was then manipulated into 0.7 or 1.0 mm quartz capillaries, always in the presence of the ethanolic solution. Capillaries were sealed at one end with a micro-torch and at the second end with jewelers wax.

X-ray patterns were obtained with a Searle camera using toroidal optics

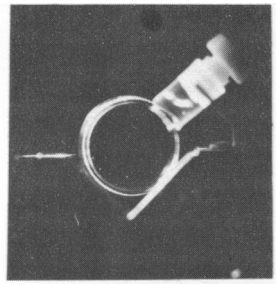
and nickel-filtered CuK_α radiation. X-rays were generated by a GX18 rotating-anode X-ray generator (Elliott Brothers, Ltd.), operating at approximately 35 kv and 50 mA with a 100 μ focusing cup. The camera was flushed with helium to reduce air scattering. Patterns were recorded on either Ilford Industrial G film or Kodirex film. The specimen-to-film distance (6.5 to 7.5 cm) was determined for each pattern from the 3.029 Å reflection of calcite placed on the outside of the specimen capillary. Measurements of spacings were made on a Stoe light box with a moveable crosshair and scales graduated in 0.1 mm. Range of error in measurement of spacings of crystalline reflections is ±0.2 (at wide angles) to ±0.3 Å (at small angles), most of which is caused by uncertainty in the specimen-to-film distance of the calcite relative to the sample.

Techniques for the spectral measurements have been described.[9] Nucleic acid concentrations were determined from measured optical densities in the absence of ethanol. Extinction coefficients at 260 nm for calf thymus DNA, T2 DNA, poly[d(A-C)·d(G-T)], and poly[r(A-C)·r(G-U)] were taken to be 6450,[10] 6464,[11] 6500,[12] and 6900.[6]

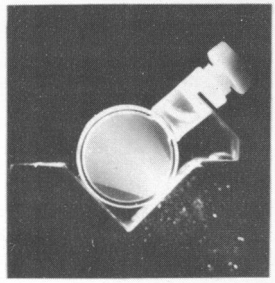
RESULTS

Differences in the visual appearance of calf thymus DNA (Miles) precipitated from 60% and 80% ethanol by the addition of NaCl are seen in Figure 1. At 60% ethanol, this DNA forms a fine precipitate that only slowly settles out of suspension. On the other hand, the precipitate formed from 80% ethanol is flocculent and immediately begins to sediment.

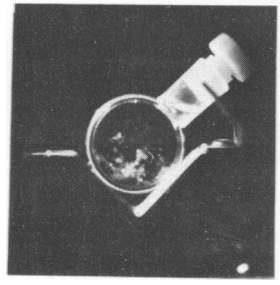
We measured the CD and absorption changes that occur as calf thymus DNA is precipitated from 60% ethanol by the addition of NaCl. CD spectra were taken in 1, 2, and 10 cm path length cells for DNA at concentrations of 25, 12.5, and 2.5 μg/ml, respectively, to determine whether the spectra obtained differed with DNA concentration. The results are shown in Figure 2. The spectra obtained with the highest DNA concentration show less noise and more detail at low wavelengths. The lowest DNA concentration approaches that used for electron microscopic studies of DNA condensation, to which we will refer in the discussion. Upon raising the salt concentration to 5 mM Na^+, all the samples showed similar spectral changes, independent of DNA concentration, the most noticeable change being an increase in the positive CD contributions above 230 nm and a concomitant shift of the crossover to 256-257nm. The spectra shown at 5 mM in Figure 2 were taken 1 hr after salt was added and were not different from those taken



(a)
60% ethanol
0.7 mM Na^+



(b)
60% ethanol
25 mM Na^+



(c)
80% ethanol
5 mM Na^+

FIGURE 1 - Photographs of calf thymus DNA solutions in a cylindrical 1 cm cell (side-lighted). (a) Unprecipitated DNA in 60% ethanol; (b) DNA precipitated in 60% ethanol; (c) DNA precipitated in 80% ethanol. In this and other figures the ethanol concentration is given in weight percent.

immediately. This was true regardless of the DNA concentration.

As the Na^+ concentration was raised to between 5 and 10 mM, the absorption spectra of the samples in 60% ethanol showed a slight increase at long wavelengths due to light scattering as the DNA began to condense visibly. The absorption spectra of the sample at 25 μg/ml are shown in Figure 3 (top panel). The CD spectra of all the samples at 10 mM Na^+ showed the appearance of a large negative long-wavelength tail due to

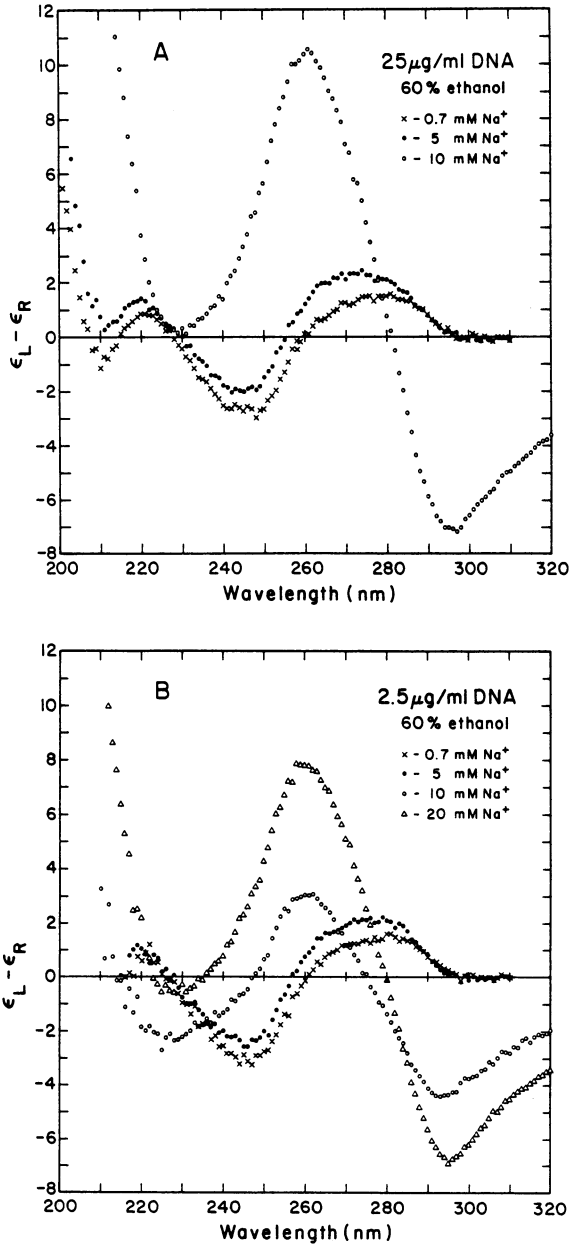


FIGURE 2 - CD spectra of calf thymus DNA in 60% ethanol at increasing salt concentrations. (A) 25 μg/ml DNA in a 1 cm cell.
(B) 2.5 μg/ml DNA in a 10 cm cell.

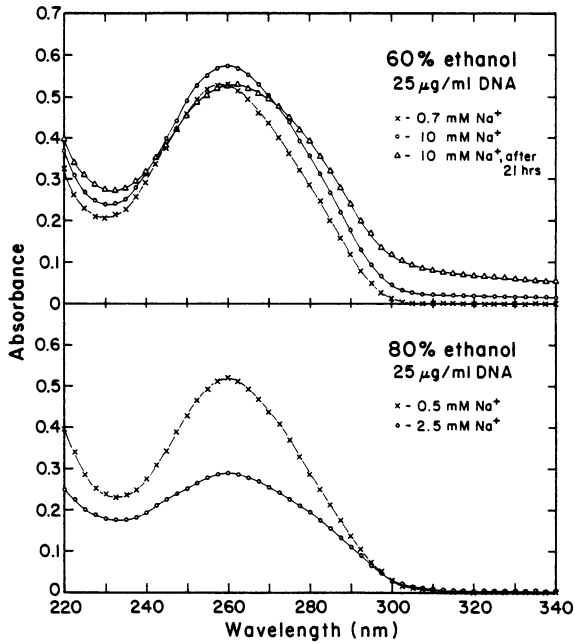


FIGURE 3 - Absorption spectra of calf thymus DNA in 60% and 80% ethanol at increasing salt concentrations.

differential light scattering (Figure 2). In addition, positive CD bands appeared at 260 nm and at wavelengths lower than 225 nm. These spectral features developed more slowly for DNA at the lowest concentration, but could be accelerated by the addition of more salt. Therefore, it is likely that intermolecular aggregation of molecules contributes to these spectral features.

In 80% ethanol (0.5 mM Na^+), prior to precipitation by added salt, calf thymus DNA is in the A conformation and has a non-conservative CD spectrum with a large positive band at 270 nm.[5,6] This differs significantly from the CD spectrum of DNA in 60% ethanol at low salt; compare Figures 2 and 4. The DNA precipitates at lower salt concentrations (1-2.5 mM Na^+) when in 80% ethanol then when in 60% ethanol. The spectra of DNA precipitated from 80% ethanol by added salt are characteristically different from those that occur upon precipitation of DNA from 60% ethanol. Figures 3 (bottom panel) and 4 show the absorption and CD changes upon precipitation of calf thymus DNA (Miles) from 80% ethanol. Although the resulting floc-

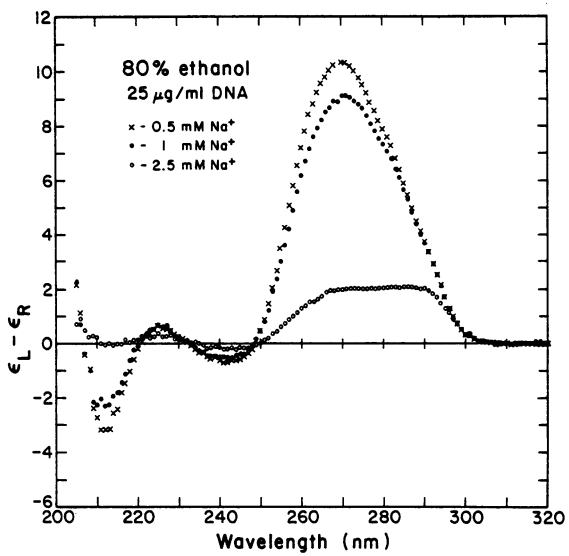


FIGURE 4 - CD spectra of calf thymus DNA in 80% ethanol at increasing salt concentrations.

culent precipitate in 80% ethanol does not provide a uniform sample in the beam, and the CD and absorption spectra are subject to large fluctuations, we never observed spectra other than those of the A conformation as the DNA precipitated in 80% ethanol.

We were interested to see whether T2 phage DNA, which has 75% of its hydroxymethylcytosine residues glucosylated,[13] could form the same precipitate as calf thymus DNA from 60% ethanol. Presumably because of its glucosylation, T2 DNA is prevented from adopting the A conformation in fibers.[14] Nevertheless, it can precipitate from 60% ethanol into the same form as calf thymus DNA, as seen in Figure 5. Therefore, the precipitated form of DNA from 60% ethanol apparently does not require the A conformation. (The CD spectrum of T2 DNA in 80% ethanol can show an increased positive 275 nm band, as reported by Girod et al.[8] However, this "A" spectrum has only one-half the magnitude of the major positive band seen for calf thymus DNA in the A conformation; also, this band exhibits unusual melting behavior which, as discussed by Girod et al.,[8] may be related to tertiary, rather than secondary, structural changes. Thus, it has not been shown that T2 DNA can exist in a true A secondary conformation in solution.)

Formation of the precipitate from 60% ethanol is readily reversed by

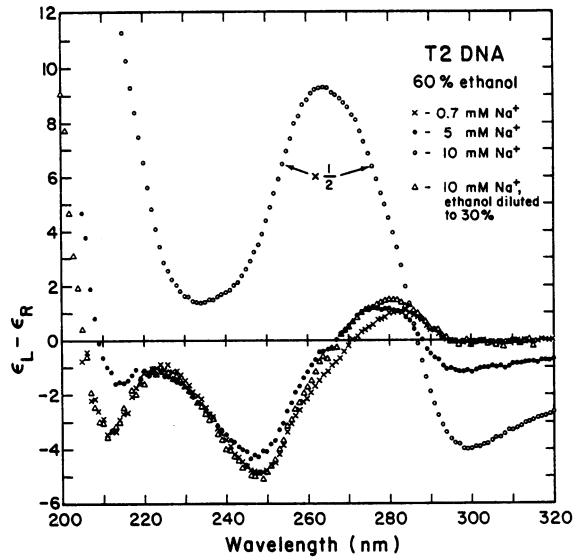


FIGURE 5 - CD spectra of sonicated T2 phage DNA in 60% ethanol at increasing salt concentrations. Also shown is a spectrum of the DNA after dilution of the precipitate into 30% ethanol. The spectrum marked "x ½" has twice the magnitude shown on this ordinate scale.

dilution of the ethanol to a lower concentration, as is shown in Figure 5 for T2 DNA. The precipitate formed by calf thymus DNA from 60% ethanol also readily reverses upon dilution.

Synthetic poly[d(A-C)·d(G-T)] forms the same type of precipitate as observed for natural DNAs at ethanol concentrations below which a B to A conformational transition occurs. The spectra are shown in Figure 6A. (These spectra were obtained with the DNA at 55% ethanol since this DNA is in the A conformation at 60% ethanol.[6]) On the other hand, we have been unable to form such a precipitate from the corresponding synthetic RNA polymer, poly[r(A-C)·r(G-U)]. Under similar conditions used to precipitate the synthetic DNA, the RNA precipitates as a flocculent mass and the CD spectra are variable, although spectral features of the A conformation are always present, as in Figure 6B. Since RNA is always in an A conformation and apparently cannot form the same precipitate as DNA at ethanol concentrations below 60%, these data support the notion that the DNA precipitate formed at 55% ethanol contains molecules in the B family of secondary conformations.

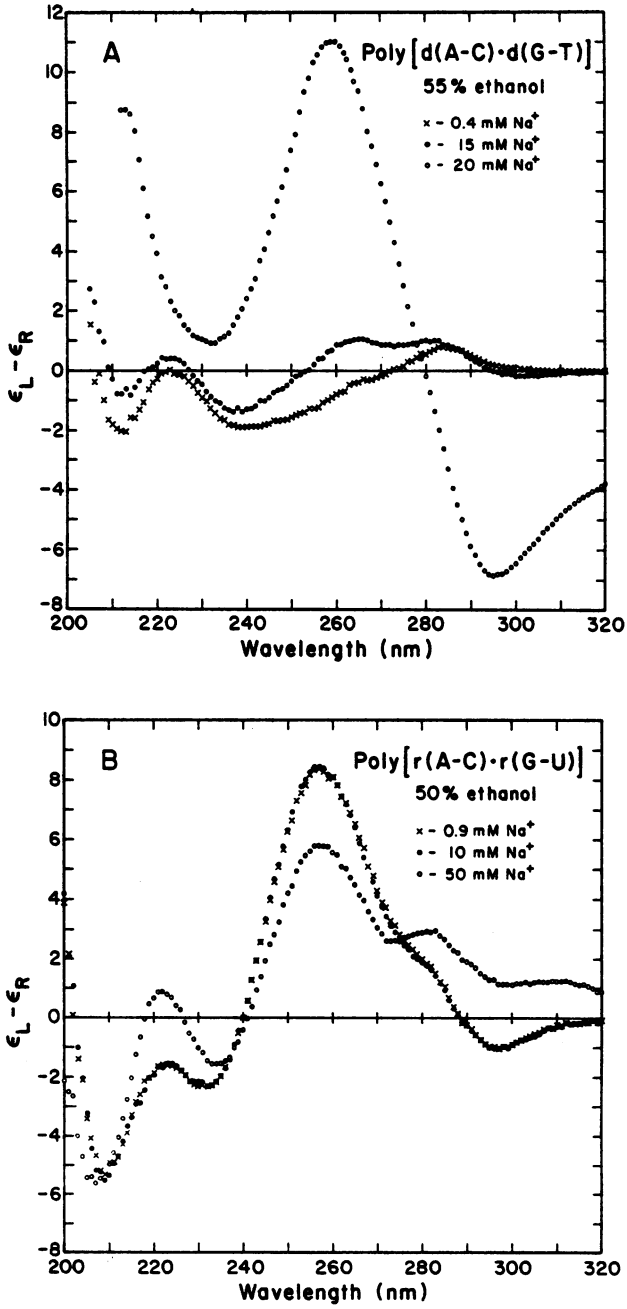


FIGURE 6 - CD spectra of a synthetic DNA and RNA in ethanol at increasing salt concentrations.

A final CD experiment provides additional support for this interpretation. It is known[5] that divalent ions inhibit or prevent the B to A conformational transition in ethanol. Notwithstanding, calf thymus DNA will form a precipitate with the same spectral features as those shown in Figure 2 in the presence of small amounts of Mg^{++}, even at 30% ethanol, as shown in Fig. 7.

The above experiments with T2 DNA, the synthetic polymers, and calf thymus DNA in the presence of Mg^{++} all indicate that the fine precipitate of DNA induced by salt from 60% ethanol contains molecules in the B conformation and differs from the precipitate formed by molecules in the A conformation. Nevertheless, these data alone do not exclude that DNA molecules undergo a change in secondary conformation to the A conformation simultaneous with their precipitation from 60% ethanol and that the positive band at about 260 nm in the CD spectra of precipitates from 60% ethanol arises from the major positive band of molecules in the A conformation. Therefore, we proceeded to obtain X-ray patterns from both types of precipitate to determine directly the DNA secondary conformation in each.

For X-ray diffraction, precipitates were formed in dilute solution (about 30 µg/ml in 60% ethanol and 14 µg/ml in 80% ethanol) so that CD spectra could be obtained on parallel samples. The clearest X-ray patterns were obtained from samples of calf thymus DNA of molecular weight 10-12 x 10^6 from Boehringer-Mannheim, and the best representatives of these are shown in Figures 8(b) and 8(c). For comparison, a pattern of unpreci-

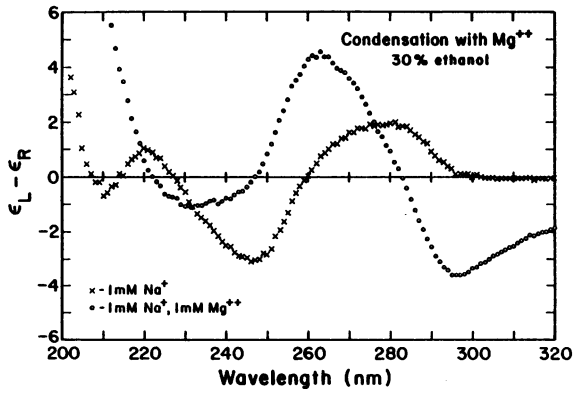


FIGURE 7 - CD spectra of calf thymus DNA precipitated in 30% ethanol by the addition of $MgCl_2$.

pitated DNA gel in water (about 10% DNA by weight) using calf thymus DNA from Miles is shown in Figure 8(a). The latter pattern represents the molecular DNA transform and does not contain crystalline reflections as do the patterns of the condensed DNA samples. The diffuse rings at 13.4 ±0.4 Å and 8.4 ± 0.3 Å are the same as reported by Maniatis et al.[15] for calf thymus DNA at similar concentrations; the maximum near 8.4 Å is a

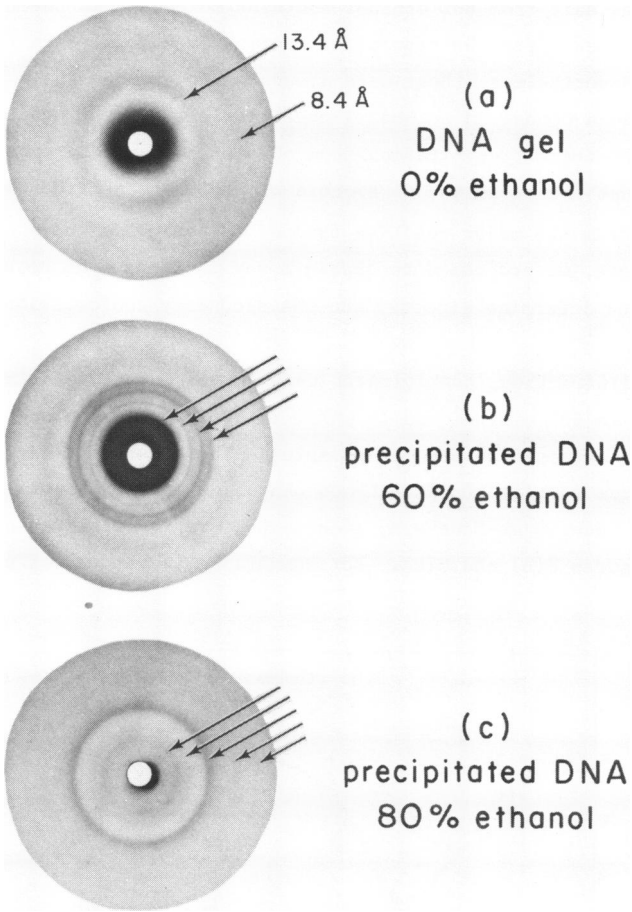


FIGURE 8 - X-ray patterns of: (a) unprecipitated DNA gel in 0% ethanol, showing diffuse rings at 13.4 and 8.4 Å; (b) DNA precipitated from 60% ethanol (the equivalent Bragg spacings of the 4 marked rings are 22.7, 15.9, 12.9, and 11.2 Å, at increasing diameters); (c) DNA precipitated from 80% ethanol (the equivalent Bragg spacings of the 5 marked rings are 21.7, 16.1, 11.4, 8.2 and 6.8 Å, at increasing diameters).

characteristic of the B conformation.[15]

X-ray patterns of precipitates from 60% ethanol showed crystalline reflections at 22.7, 15.9, 12.9, and 11.2 Å (±0.2 to ±0.3 Å). See Figure 8(b). These correspond very nicely to reflections expected for B conformation DNA packed in a hexagonal unit cell as described by Langridge et al.,[16] with unit cell dimensions of a = 46 Å and c = 34.6 Å. The 110, 102, 202, and 212 (hkl) reflections from such a hexagonal cell would occur at 23, 15.9, 13.1, and 11.4 Å, respectively. The molecules could not be in the C conformation, which packs in a hexagonal lattice of a = 35 Å and c = 31 Å.[17]

The calf thymus DNA from Boehringer-Mannheim, of molecular weight 10-12 x 10^6, precipitated from 60% ethanol as fine strands upon the addition of salt to 10 mM Na^+. Such a precipitate was used to give the pattern in Figure 8(b). The same pattern, although more faint, was obtained from DNA precipitates formed from sheared DNA (sheared by forcing the stock DNA solution at 2.3 mg/ml through a 24 gauge needle) and from precipitates of calf thymus DNA from Miles. It was more difficult to concentrate the DNA from the fine precipitate formed from these lower molecular weight samples, but the appearance of the 22.7 Å ring was always quite definite. CD spectra taken on parallel condensates from the calf thymus DNA from Miles showed CD spectra as in Figure 2, with a differential CD scattering tail.

X-ray patterns of condensates from 80% ethanol were generally easier to obtain since the flocculent precipitate could be readily collected and concentrated. As shown by Figure 8(c), these patterns were very different from those of the precipitate from 60% ethanol. Diffraction maxima could be seen at 21.7, 16.1, 11.4, 8.2, and 6.8 Å (±0.2 to ±0.3 Å). To see whether this pattern corresponded to DNA in the A conformation packed in a triclinic lattice, we estimated the equivalent Bragg reflections that would be observed for an unoriented A pattern. Since many reflections overlap in the oriented DNA A pattern, we estimated the distance to the center of each close group of reflections (see ref. 14, plates I and IV, for patterns from fibers at 75% relative humidity). We obtained equivalent Bragg distances of 20.8 (equitorial group closest to the center), 16.3 (closest group on 1st layer line), 11.1 (closest group on 2nd layer line, plus second group on equator - this should give an intense ring), 8.1 (strongest reflection of second group on 2nd layer line) and 6.7 Å (third group on 2nd layer line). The overall correspondence of these estimated distances with our measured

values and the qualitative agreement of the intensities shows that DNA precipitated from 80% ethanol remains in the A conformation.

X-ray patterns obtained from DNA condensed from 80% ethanol often contained extra reflections of salt precipitated with the DNA, but these never obscured the three innermost rings due to the DNA, which were generally very clear. The pattern shown in Figure 8(c) was obtained from the high molecular weight Boehringer-Mannheim sample of calf thymus DNA, which precipitated in 80% ethanol in the presence of only 0.34 mM Na^+ (phosphate).

DISCUSSION

We have presented experimental evidence that DNA precipitated from ethanolic solutions by the addition of salt maintains the secondary conformation in the precipitate that it had in solution. Although precipitates formed from DNA in the B conformation (from 60% ethanol) and the A conformation (from 80% ethanol) both contain molecules packed in regular crystalline arrays, there are two major differences: (1) The visual appearance of the precipitate from 60% ethanol has a fine particulate nature, unlike the flocculent precipitate formed from 80% ethanol (Figure 1). Even when the DNA has a rather large molecular weight (10-12 x 10^6), the precipitate from 60% ethanol has a thin stringy nature and can readily be distinguished from that formed from 80% ethanol. (2) The CD spectra of precipitates from the lower ethanol concentrations show evidence of unusual long-range order in that the spectra have large magnitudes and a differential-scattering tail that extends to long wavelengths (Figures 2,5,6 and 7). Our present results show that the positive band near 260 nm in these spectra is not related to the A secondary conformation. When the CD of such precipitates is completely corrected for differential scattering by fluorescence-detected circular-dichroism measurements (Reich *et al.*[18]), the magnitude of the positive band at near 260 nm actually increases in magnitude, indicating that the intrinsic CD of individual molecules in the B conformation also does not dominate such spectra and that there must be a large CD contribution from the tertiary structure. This is unlike the DNA precipitated from 80% ethanol for which the CD spectra are very much dominated by the intrinsic CD of the molecules in the A conformation. (We have found in other experiments that if DNA is first precipitated in 60% ethanol plus salt, and ethanol is then added to 80%, the CD spectrum of the precipitate in 80% ethanol retains spectral features of the precipitate first formed in 60% ethanol. Thus, the pres-

ence of 80% ethanol may not assure a secondary conformational change of DNA to the A conformation; when sufficient salt is present, molecules may precipitate in the B conformation at lower ethanol concentrations and remain in the B conformation as the ethanol concentration is raised.)

The salt-precipitated form of DNA from 60% ethanol differs from the polymer and salt-induced ψ condensate first reported by Jordan et al.[19] Although the ψ condensate contains molecules in the B secondary conformation, there is no evidence of hexagonal crystalline packing from X-ray scattering studies,[15] and CD spectra of ψ-condensed DNA show only large negative bands with minimal differential scattering at long wavelengths.[19] Instead, the particulate nature of the salt-precipitated form of DNA from 60% ethanol may be another expression of the tendency of DNA to pack into "Giannoni crystals".[20] Most recently studied by Lerman et al.,[21] these hexagonal crystals of DNA can be formed from DNA of low molecular weight by slow cooling in 34% ethanol by weight. X-ray diffraction patterns from such crystals show reflections at spacings mostly indistinguishable from those we observe.[20,21] Thus, we believe that DNA in the B conformation may condense from solution into two forms: (1) the ψ condensate with large negative CD bands, and (2) Giannoni-like crystallites with large positive CD bands in the region of the ultraviolet absorption bands and with a negative differential scattering tail.

This conclusion is in agreement with results recently published by Damaschun et al.[22] These authors found that two types of DNA-spermine complex can be formed, upon varying the NaCl concentration, that give either large positive or large negative CD bands. Their X-ray diffraction data showed that DNA is in the B conformation in both types of complex and that, moreover, the complex with large positive CD bands is crystalline. Our results indicate that the two types of complex studied by these authors may be related to the inherent ability of DNA in the B conformation to condense into different forms. We agree with the conclusion of these authors that the appearance of large positive CD bands does not necessarily indicate the presence of the DNA A conformation. In other work, Shin and Eichhorn[23] have found that the CD spectra of DNA-polylysine complexes can undergo changes from large negative to large positive values upon metal ion binding. Such a CD change could be caused by the formation of DNA crystallines, instead of by a B to A change in DNA secondary conformation as proposed by these workers.

Whether DNA in the A conformation can also be condensed into a form that exhibits large CD bands due to tertiary structure remains an open question.

Evdokimov et al.[24] have reported that the formation of ψ condensates with double-stranded RNA results in the appearance of large positive CD bands.

Our results have several implications for the interpretation of ethanol-condensed forms of individual DNA molecules by electron microscopy. Lang[25] originally recognized that individual DNA molecules can be compacted into regular tertiary structures by ethanol-dehydration. This finding was of importance for electron microscopy of DNA containing specimens that involved ethanol dehydration during preparation. Subsequent studies[26,27] have resulted in apparent disagreement as to whether the most compact structures of individual DNA molecules, formed from 95% ethanol by volume, are supercoiled or folded structures. While our present results do not finally resolve this disagreement, our finding that the precipitates formed from the B and A secondary conformations can differ does show that a new variable must be considered in designing and interpreting the electron microscopy experiments. It is quite possible that the B and A secondary conformations of DNA will form different compact structures upon condensation in ethanol. If so, differences in the precise manner in which ethanol dehydration plus condensation is performed could result in the formation of different structures and an apparent disagreement in data. We have undertaken experiments designed to explore this possibility. In preliminary electron microscopy experiments we have indeed found that double-stranded RNA from reovirus does not readily form compact particles like those of DNA under identical conditions. (RNA is constrained to the A family of secondary conformations.)

Another implication of our present results for the interpretation of ethanol-condensed individual molecules is that the supercoiling of individual molecules probably begins with the molecules in the B conformation. There is agreement by various workers that DNA molecules have the ability to form supercoiled (coiled-coil) structures upon ethanol dehydration.[25-27] Supercoiled structures can be formed at ethanol concentrations well below that of 70-80% needed to cause a B to A conformation transition.[25,26] Individual DNA molecules are condensed into supercoils at DNA concentrations of 0.2 to 2 μg/ml when studied by electron microscopy. When we precipitate DNA from 60% ethanol at concentrations as low as 2.5 μg/ml we find that the CD spectrum has characteristics we now associate with crystalline packing of molecules in the B conformation (Figure 2B). As we have previously reported,[28] CD measurements have been performed on DNA (linear PM2 phage DNA) condensed from solutions containing 0.2 M NH_4^+ by the addition of ethanol, under conditions similar to those used to obtain supercoiled molecules as seen by

electron microscopy. We found that also under these conditions the DNA precipitated to give the spectral features shown in Figure 2 (see Figure 1, ref. 28). Moreover, we have obtained X-ray patterns for DNA condensed from 60% ethanol using NH_4^+ as the anion. In one experiment, ammonium acetate was added to precipitate DNA in 60% ethanol and a crystalline pattern as in Figure 8(b) was obtained. In another experiment, a DNA solution containing 0.2 M ammonium acetate was precipitated by adding ethanol to give 60% by weight. The precipitate showed a non-crystalline B pattern, as in Figure 8 (a). In both cases, the DNA remained in the B conformation. All of these data indicate that the condensation of individual DNA molecules into supercoiled forms begins with the molecules in the B conformation. (There is no evidence from our X-ray diffraction patterns that our precipitated samples of DNA contain supercoiled molecules; in fact, the packing of molecules into crystalline lattices similar to those seen for fibers argues against supercoiling of either the B or A DNA conformations in our precipitates. However, this does not argue against supercoiling as a means by which an individual molecule condenses upon itself.)

Support for the notion that DNA in the B conformation can become condensed into a supercoiled self-structure is provided by energy calculations by Levitt.[29] Although preliminary, these calculations indicate that smooth bending is more difficult for DNA in the A conformation than for DNA in the B conformation. In addition, other workers have found that DNA is in the B conformation in nucleosomes as indicated by Raman spectroscopy,[30] in particles formed by condensation of DNA with polyamines as indicated by CD spectroscopy,[31] and packed in phage P22 as indicated by X-ray diffraction.[32]

CONCLUSION

Our experiments have shown that DNA precipitated by ethanol plus salt stays in the B conformation at 60% ethanol and stays in the A conformation at 80% ethanol during the course of precipitation. The unusual CD properties of the DNA precipitates formed from 60% ethanol are very likely related to the packing of molecules into Giannoni-like crystalline arrays. These results are of general importance for the design and interpretation of experiments to study the tertiary structures of condensed DNA, including structures studied by electron microscopy. Also, DNA isolation procedures that involve ethanol-induced precipitation should be performed with these results in mind, since fine precipitates formed in 60% ethanol from low molecular weight DNA in the B conformation may be difficult to recover,

resulting in reduced yields compared with precipitates formed in 80% ethanol. Finally, it is tempting to conclude from our present study, together with other recent work, that one role of the DNA B conformation may be to allow DNA to form compact tertiary structures such as supercoils not readily available to RNA.

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