CONFORMATION OF DNA IN ETHYLENE GLYCOL

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Received September 2, 1970

SUMMARY

Detailed circular dichroism studies lead us to propose that the low temperature form of DNA in ethylene glycol is the C type conformation. We confirm that DNA in high salt is intermediate between B and C form and suggest that the high temperature form of DNA in ethylene glycol is a "random coil". Speculation on C form DNA as an important *in vivo* conformation is given in the conclusion. The DNA-ethylene glycol system offers a convenient way to study this structure.

INTRODUCTION

The effects of an ethylene glycol environment of moderate ionic strength on the conformational properties of DNA have been investigated in several laboratories (1-6) utilizing optical techniques. Duggan (1) and Eliasson et al. (2) first noted the capability of DNA to retain an ordered secondary structure when dissolved in glycol-salt. They found a cooperative thermal transition for DNA occurring around room temperature. Luzzati et al. (3) examined the problem using X-ray scattering techniques, and found the DNA to be rod-like with axial radius of gyration and mass per unit length consistent with the B form of DNA below the melting temperature (T_m). Above the T_m, DNA assumed another rod-like form with half the mass per unit length. Luzzati et al. (3) tentatively proposed the high temperature form to be either single stranded stacked or a novel intercalated form.

Green and Mahler (4,5) have extensively investigated the conformation assumed by DNA and synthetic polynucleotides in glycol containing .05M KF and 1.0 mM EDTA (EG) using absorption, optical rotatory dispersion (ORD) and circular dichroism (CD) spectroscopy. They have noted complete reversibility between the form in EG and B form DNA in aqueous solution. Their low

temperature CD spectrum in EG is particularly interesting, exhibiting a complete loss of the positive rotational strength at 276nm found for aqueous DNA, coupled with a broadening and intensification of the negative band at 241 nm. Green and Mahler (4,5) interpret the low temperature, nonconservative CD spectrum as due to a DNA conformation with some similarity to the B form, but with the bases tilted with respect to the helix axis. However they are unable to offer a specific model.

Here we present a more detailed CD spectrum of DNA in EG which reveals three CD bands not previously reported. A comparison of this CD spectrum with spectra of unoriented DNA films recently measured by Tunis-Schneider and Maestre (7) allows us to propose that DNA in EG is the C form (8).

MATERIALS AND METHODS

Highly polymerized calf thymus DNA was obtained from Worthington Biochemicals. This DNA gave the same low temperature CD spectrum in EG both before and after purification to remove protein and RNA. To make up a stock solution of approximately 0.25 mg/ml it was dissolved at 4°C in ethylene glycol containing 0.05M KF and 1.0 mM EDTA. Aliquots were then diluted with appropriate solvents to give a system with 0.05M KF and 1.0 mM EDTA and an optical density of approximately 1.0 at 260nm. The concentrations of the DNA solutions, on a mononucleotide basis, were determined spectrophotometrically using an E_P^{max}=6600 for the aqueous DNA solutions and an E_P^{max}=6790 for the glycol DNA solutions as is consistent with work in other laboratories. CD spectra were measured on a Model CD-SP Durrum-Jasco circular dichroism recorder.

RESULTS AND DISCUSSION

The CD spectrum of DNA in EG at 25°C reported by Green and Mahler (4,5) reveals a complete loss of dichroism above 280 nm and a negative dichroic band with $\varepsilon_L - \varepsilon_R = 2.5$ centered at 241 nm. Similar results have been obtained for other types of DNA (4-6). Our spectrum (Fig. 1) taken at 20°C in the same

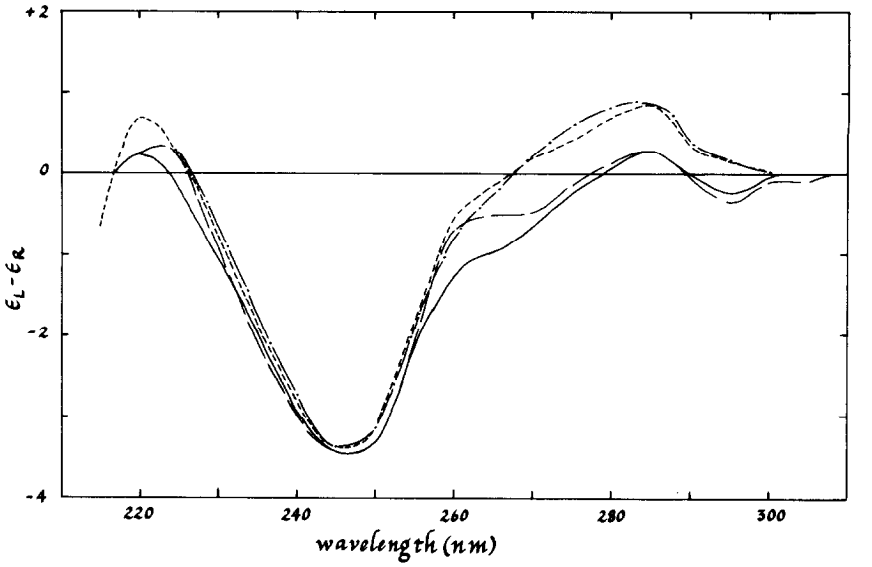

Fig. 1. Comparison of CD of DNA in EG at 20°C (____) to LiDNA film in C form from Tunis-Schneider and Maestre (7) (_ _); and DNA in 25% (v/v) water-EG at 20°C (—·—) to DNA in water with 6M NaCl, also from Tunis-Schneider and Maestre (7), (----).

medium exhibits considerable detail, in addition to the large negative band which we observe at 246nm with $\epsilon_L - \epsilon_R = 3.5$. We also find a small conservative dichroism with a crossover at 290nm, a negative shoulder at 265nm, and a small positive maximum at 220 nm.

Tunis-Schneider and Maestre (7) have recently taken CD spectra of unoriented DNA films as a function of counterion type, concentration, and relative humidity. By varying these parameters they have been able to observe transitions between the various forms of DNA in analogy with the transitions found in fibers. This study is extremely important, since they are able to relate the geometrical structures of DNA as determined by X-ray work on fibers to conformational dependent CD spectra. A comparison of Figure 1 reveals that our spectrum of DNA in EG is strikingly similar to their spectrum of LiDNA at 75% relative humidity. In analogy with fiber work Tunis-Schneider and Maestre (7) identify their film as C type DNA (8) with 9 1/3 bases per turn, 31Å pitch, and 6° base tilt.

The discrepency between the X-ray scattering work (3) which indicates DNA in the B form in low temperature glycol and the CD work which indicates a structure other than B form is now resolved. The C form would, as the B, give rod-like scattering curves and would have about the same mass per unit length as the B form.

Green and Mahler (4) have noted the similarity of their low temperature ORD curves for DNA in EG to those reported by Tunis and Hearst (9) for DNA in concentrated salt solutions. Both conditions show reversibility of an induced conformational change upon return to aqueous environment where the DNA assumes the B form. Tunis-Schneider and Maestre (7) present the CD of DNA in high salt which we compare with our spectrum of DNA in 25% (v/v) water-EG in Figure 1. The similarity of the spectra confirms their suggestion that DNA in high salt is in a conformation intermediate between B and C forms.

Figure 2 presents the CD of DNA in EG as a function of temperature which has not previously been reported. It is apparent that a thermal transition occurs with an increase in positive rotational strength at 278 nm. Plotting

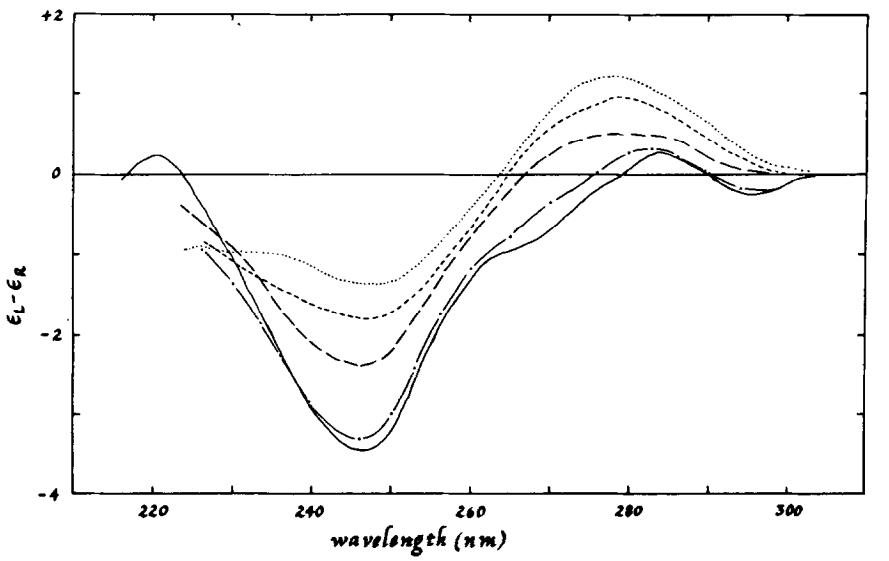

Fig. 2. CD of DNA in EG at various temperatures: (——) at 20 and 25°C; (—·—) at 30°C; (— —) at 33°C; (----) at 35°C; (····) at 40, 50 and 70°C.

$\varepsilon_L - \varepsilon_R$ for 278 nm versus temperature gives a T_m of 33°C. The high temperature conservative spectrum is similar to that of denatured DNA in aqueous solution (10). This observation is in contrast to the results of X-ray scattering (3).

CONCLUSION

We propose that the low temperature form of DNA in EG is the C type conformation analogous to the form found in fibers of LiDNA at low humidity (7,8). This seems reasonable since our detailed CD spectrum of this system is so similar (Fig. 1) to the spectrum of C form DNA films measured by Tunis-Schneider and Maestre (7).

The reversible transition in DNA conformation occurring on going from glycol to aqueous medium (4) can be labeled as a C to B form transition by analogy with the reversible dehydration-rehydration results in LiDNA films (7). In addition, our CD spectrum of DNA in 25% water-EG compares with that of DNA in high salt confirming the suggestion that DNA in high salt is on its way to the C form (7).

Our study of the CD and DNA in EG as a function of temperature gives a $T_m = 33°C$. We suggest that the high temperature form is simply a "random coil" since its CD compares with that of high temperature DNA in aqueous solution (10).

Previously C form DNA was considered to be a novel and rare occurance since it only appeared in diffraction work on specially treated fibers of DNA with Li counterion (8). However, in glycol the C form is present with either K (4,5) or Na (1,2,3) cations. In addition, C form DNA may be an important *in vivo* structure of DNA. ORD spectra of DNA bacteriophage heads (11,12) show a loss of the positive rotation around 290 nm found for aqueous B form DNA. This same loss of rotation is found for DNA in ethylene glycol (4,5). This corresponds to more recent work where Tunis-Schneider and Maestre (7) point out that some unpublished CD data on intact T5 and T7 DNA viruses show a collapse of the 278 positive CD band. We speculate that DNA may assume the C form whenever efficient packing is necessary. In orthorhombic C form fibers each nucleotide occupies only $107A^3$ (8). This compares with about $118A^3$

required for each nucleotide in A form (13), B form (14), and hexagonal C form (8). Thus it is not surprising to find DNA in many bacteriophage heads closely packed in C form DNA. In addition, it is interesting to compare this work with that of Fasman et al. (15) on the conformational changes occurring in DNA upon binding of f-1 histone. Adding ethylene glycol to an aqueous solution of DNA produces spectral changes somewhat similar to those observed when f-1 histone is added to an aqueous solution of DNA.

The ethylene glycol system offers an easy way to study the solution properties of C form DNA.

ACKNOWLEDGMENTS

We are indebted to Dr. Tunis-Schneider and Dr. Maestre for allowing us to read their manuscript before publication. R. G. Nelson wishes to acknowledge his NDEA Title IV Graduate Fellowship and W. C. Johnson his support by Public Health Service Research Career Development Award number GM 32784 from the Institute of General Medical Sciences. This work was supported in part by National Science Foundation Grant GB 8647.

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