Secondary structure of nucleic acids in the folded chromosome from *E. coli*

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ABSTRACT

The circular dichroism of membrane-free folded chromosomes from *E. coli* was measured and analyzed. The spectrum can be explained as a simple linear combination of the individual spectra of *E. coli* RNA, and *E. coli* DNA in the B form. No contribution from A form or C form DNA was detected. There was evidently some real variation in the ratio of the two nucleic acids from preparation to preparation, but the average value was 24% RNA and 76% DNA. No significant light scattering was observed and the analyses indicated no contribution to the circular dichroism from scattering artifacts. Apparently, combining DNA, RNA, and protein into membrane-free folded chromosomes does not change the secondary structure of the DNA or RNA from that found for the free nucleic acid in the same solvent system.

INTRODUCTION

The bacterial genome of *E. coli* can be isolated from gently lysed cells by sucrose density-gradient centrifugation (1) as a compact structure called the folded chromosome. The genome can be isolated either attached to or free from membrane fragments by variation of the temperature at the time of lysis (2,3). The membrane-free chromosome is still constrained to its approximate intracellular dimensions (4). It contains about 60% DNA which is arranged in about 50 independent loops (5,6), 30% RNA, and 10% protein of which 90% is core RNA polymerase (1,2). Several distinct levels of tertiary structure have been reported for the membrane-free chromosome (5,6,7), ranging from folded and supertwisted to completely unfolded. Both the RNA (5,6,7) and the protein (7) have been implicated in the maintenance of the tertiary folding.

We were interested in determining the secondary structure of the DNA and RNA which make up these important particles. Circular dichroism (CD) is the obvious technique for such an investigation because of its sensitivity to secondary structure. Tunis-Schneider and Maestre (8) have measured the CD of films of DNA. By varying the humidity and the type of counter ion these workers produced films under the same conditions that have been used with fibers of DNA to produce the A, B, and C forms (9,10). They found a different and characteristic CD spectrum for films made with the humidity and counter ion type which would be expected to produce each of the forms of DNA. It was not possible to check the films definitively to determine the form of the DNA, because the films must be isotropic for CD work. The CD spectrum obtained for the B form DNA film is accepted because it is the same as the CD measured for DNA in aqueous solution with a moderate concentration of salt (11). The CD obtained for the A form DNA film is accepted because it is similar to the CD obtained for RNA (11). However, the spectrum obtained for DNA films under conditions where DNA assumes the C form in fibers has not generally been accepted (12). In any case, it is clear that this large change in CD spectrum corresponds to some difference in the DNA. We shall assume for the purpose of writing this paper that the characteristic CD spectrum corresponds to C form DNA.

Variation in solvent conditions have produced conformations of DNA which give all three characteristic CD spectra (11, 13-22). In addition, all three CD spectra have been observed in different tertiary structures containing DNA. CD studies of collapsed DNA in ethanol (15) show the characteristic A form spectrum. CD studies of chromatin (23) and nuclease-resistant chromatin (24) suggest that the nucleic acid in these protein-DNA complexes may adopt the high salt or C form geometry. CD has been used to show that spermidine condensed T7 DNA retains its B form secondary structure (25).

Our study concentrated on the membrane-free folded chromosomes because of their compositional simplicity. We find that the secondary structure of nucleic acid components in

the genome is the same as that found in the free solution. The secondary structure of the nucleic acids is essentially unaltered by the forces which hold the genome in the folded conformation. The low concentration of isolated chromosomes (about 0.5 A_{260} OD units/ml) required the use of 2 cm cells. At this pathlength the concentration of sucrose from the density-gradient purification prevented measurements below 236nm and a determination of secondary structure of the protein.

MATERIALS AND METHODS

Reagents:

Density-gradient grade (ribonuclease free) sucrose was purchased from Schwarz/Mann. Lysozyme (muramidase), 25,000 units/mg, and E. coli B strain DNA were purchased from Worthington Biochemical Corp. Sodium deoxycholate and polyoxyethylene 20 cethyl ether (Brij 58) were purchased from Sigma. 16S and 23S ribosomal RNAs (E. coli B236) were kindly supplied by Professor Henry W. Schaup.

Preparation of Folded Chromosomes:

The E. coli strain H560 (pol Al, endo I, thy) growing exponentially in M9 medium (26) was gently lysed by the method of Stonington and Pettijohn (1) as modified by Worcel and Burgi (5). Cells were lysed at 22°C. Cell debris was removed from the lysate by centrifugation at 3000g for 5 minutes. The membrane-free folded chromosomes were separated from the lysate by centrifugation (17,000 rpm, 30-50 minutes, 4°C, Beckman SW50.1 or SW27.1 rotors) into 10% to 30% or 10% to 45% (w/v) sucrose density-gradients containing 1M NaCl and 10mM tris-HCl, pH=8. Fractions (0.5 to 1.5 ml) were collected from the bottom of each tube through an 18 gauge needle. Chromosome containing fractions were identified by absorbance at 260nm. Peak fractions were pooled, mixed by gentle rolling and used immediately for optical studies. No attempt was made to dialyze out the sucrose because the particles deteriorate with time.

Optical Studies:

Adsorption spectra were taken at room temperature (about 22°C) using a Cary 15 spectrophotometer. Scattering estimates were based on apparent optical density at 325nm. The apparent optical density at 325nm was low (0.01 OD for OD=1.0 at 260nm)

which compares well with 0.004 OD for OD=1.0 at 260nm found for typical E. coli DNA solutions. Circular dichroism spectra were measured immediately at room temperature using a Durrum-Jasco J-10 recording spectrophotometer. The instrument was calibrated with d-10-camphorsulfonic acid (Aldrich) using $\Delta\epsilon=2.20$ at 290.5nm.

The scan rates were adjusted so that the total time at room temperature did not exceed 30 minutes. To avoid possible artifacts in the CD due to scattered light, all measurements were made with the sample cell positioned as closely as possible to the photomultiplier. Error estimates were based on repeated scans of the DNA reference spectrum. In the wavelength range above 240nm, spectra were reproducible to $\pm 1\%$.

Structural Integrity of the Folded Chromosomes:

A sedimentation analysis was done using tritium labelled chromosomes to insure that the sample had not degraded during the CD measurements (7). A sample for which the CD had been measured in the usual way was compared with an aliquot of the same preparation which had been kept on ice. No change in sedimentation coefficient was observed.

To label the chromosomes (methyl- ^3H) thymidine (New England Nuclear; 40 to 60 Ci/mmol; 0.05 mCi/40 ml culture medium) was added to bacteria growing exponentially in M9 medium supplemented with 40 $\mu\text{g/ml}$ thymine. Growth was continued about one-half cell generation. Chromosomes isolated by this method had a specific activity of roughly 180,000 cpm/ μg DNA.

Spectral Analysis:

CD spectra of the folded chromosome were analyzed by the vector projection method of Baker and Isenberg (27). This procedure determines the amplitudes of the reference spectra by projecting the experimental curve on the reference spectra. This is easily done in practice by computing reciprocal functions of the reference spectra and taking the scalar product of the reciprocal functions with the experimental curve. The method uses several stringent criteria to evaluate the proper choice of reference spectra.

The reference CD spectra for E. coli ribosomal RNA and E. coli DNA were measured in the same solvent system used for

the folded chromosomes (27% sucrose, 1M NaCl, 10mM tris-HCl, pH=8). The CD spectra of 16 and 23S ribosomal RNA are nearly identical. The reference CD spectra attributed to A form DNA and C form DNA were taken from previous work in nonaqueous solution (15).

The extinction coefficient for absorption at the maximum (257nm) for E. coli DNA in 10mM sodium phosphate, pH=8 was taken to be 6700 (28). The extinction coefficient at the absorption maximum (257nm) for 16 and 23S ribosomal RNA in 100mM KCl, 50mM tris-HCl, pH=7 was taken to be 8086 (29). The extinction coefficients in 27% sucrose, 1M NaCl, 10mM tris-HCl, pH=8 were then determined by a double dilution experiment. This gave 6940 for the E. coli DNA and 8370 for the E. coli ribosomal RNA. The extinction coefficient for the folded chromosomes at the absorption maximum (257nm) was taken to be the appropriate weighted sum of the extinction coefficients for the DNA and the RNA.

Digitalization, plotting, and all calculations were done on an HP9821A calculator with its standard accessories.

RESULTS AND DISCUSSION

The mean CD spectrum of five preparations of membrane-free folded chromosomes is shown in Figure 1. One might expect a preparation at such high molecular weight particles to scatter light strongly. We observed relatively little light scattering (about 0.01 apparent absorption units at 325nm per OD unit at 260nm). In addition, our straight forward analysis presented below indicates that we were not troubled by scattering artifacts.

The reference CD spectra used to analyze the spectrum of the folded chromosome are given in Figure 2. The CD of E. coli DNA and 23S ribosomal RNA were measured in 27% sucrose, 1M NaCl, and 10mM tris-HCl, pH=8, the same conditions under which the folded chromosomes were measured. The intensity of the 280nm band of DNA under these solvent conditions is about 15% lower than that found for E. coli DNA in aqueous solution under moderate salt conditions. The decrease can be completely attributed to 1 M NaCl and is a well known effect in high salt solutions (17). Under these conditions the CD spec-

Figure 1: Analysis of CD of *E. coli* folded chromosome. Mean CD spectrum of folded chromosome (—). Contribution (24%) of RNA reference spectrum (---). Contribution (76%) of DNA reference spectrum (.....). Sum of contributions (-.-.-).

Figure 2: CD basis spectra for analysis. B form DNA (—), 23S ribosomal RNA (---), A form DNA (.....), C form DNA (-.-.-).

trum is only slightly changed and the *E. coli* DNA can be considered to be in the B form for all practical purposes. The reference CD spectrum attributed to A form DNA is that of DNA in 80% ethanol. The CD spectrum of DNA in 95% methanol is attributed here to C form DNA.

Table I: Projection Analysis of Folded Chromosome

<u>Range (nm)</u>	<u>% B form DNA</u>	<u>% RNA</u>	<u>% A form DNA</u>	<u>% C form DNA</u>	<u>Total</u>
236.0-339.2	76.6	23.7	-	-	100.3
260.0-339.2	76.1	23.6	-	-	99.7
260.0-289.6	76.4	23.5	-	-	99.9
249.6-300.0	75.8	23.7	-	-	99.5
236.0-339.2	77.6	25.2	-1.1	-	101.7
260.0-339.2	81.1	24.9	-1.6	-	104.4
260.0-289.6	76.5	23.6	0.0	-	100.1
249.6-300.0	75.5	23.5	0.2	-	99.2
236.0-339.2	71.5	26.5	-	6.6	104.6
260.0-339.2	73.9	25.8	-	7.1	106.8
260.0-289.6	72.5	27.1	-	10.9	110.4
249.6-300.0	77.0	22.9	-	-2.0	97.9

Table I summarizes the pertinent results of our analysis using the method of Baker and Isenberg (27). Several stringent criteria are available in this method to evaluate the choice of reference spectra. First, the amplitude of the reference spectra should be non-negative and sum to 100%. Second, the amplitudes of the various reference spectra should be independent of the choice of wavelength range. Third, additional reference spectra which are not contained in the experimental data will give a zero amplitude if they are linearly independent of the other reference spectra.

The experimental spectrum can be successfully analyzed by using only the RNA and B form DNA reference spectra, as the first part of Table I shows. The second part of the table indicates that A form DNA does not contribute to the experimental spectrum. Adding C form DNA as a third reference spectrum (last part of Table I) produces an analysis which violates the first and second criteria. Other combinations of the reference

spectra were used for the analysis but they all failed in one or more of the criteria. In particular, it was interesting to find that the spectrum attributed to A form DNA would not substitute for the spectrum of RNA, even though the two reference spectra are similar.

The results presented in Table I indicate that the folded chromosome CD spectrum is a simple linear combination of the CD spectra of E. coli DNA and RNA. The resolution of the average experimental spectrum into these two reference spectra as well as the CD spectrum calculated from the analysis are given in Figure 1. The CD reference spectra attributed to A form DNA and C form DNA are not necessary to analyze the experimental spectrum.

Each of the five CD spectra which were averaged to give the spectrum of the folded chromosome in Figure 1 were analyzed individually. Each spectrum could be explained as a linear combination of the spectra of E. coli RNA and E. coli DNA in the B form. Amplitudes ranged from 20% RNA and 80% DNA to 30% RNA and 70% DNA. This variation exceeds experimental error and evidently reflects a real compositional variation from preparation to preparation of folded chromosomes. The average experimental CD spectrum and its analysis is similar to the results for the typical CD spectrum.

Previous workers have estimated the composition of the nucleic acid portion of the membrane-free folded chromosomes of E. coli as 30% RNA and 70% DNA. This compares favorably with our average values of 24% RNA and 76% DNA.

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