JOURNAL OF BACTERIOLOGY, Apr. 1971 p. 238-242

Vol. 106, No. 1
Printed in U.S.A.

Asymmetric Distribution of Guanine Plus Thymine Between Complementary Strands of Deoxyribonucleic Acid of Members of the *Enterobacteriaceae*

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Received for publication 7 January 1971

Analysis of the deoxyribonucleic acid prepared from *Proteus mirabilis*, *Escherichia coli*, and *Serratia marcescens* in an alkaline CsCl gradient has shown that there is an asymmetric distribution of guanine plus thymine residues between the complementary strands of the deoxyribonucleic acid.

The chromosomal deoxyribonucleic acid (DNA) prepared from a variety of different bacterial species has been found to form a unimodal and approximately symmetrical band when examined in a CsCl density gradient (9, 10, 17). The distribution of DNA in the gradient is indicative of a relatively narrow spread in the DNA base composition about the mean value. The modal density of the DNA from different sources is a linear function of the mean base composition of the DNA. In a CsCl gradient at neutral *p*H, denatured DNA has an increased buoyant density and usually only a single DNA band is observed in the density profile of the DNA. A much larger increase in the density of denatured DNA is observed in an alkaline CsCl gradient (19). Under these conditions guanine (G) and thymine (T) residues of DNA strands are titrated. The larger increase in the density of the DNA is due to the binding of Cs^+ ions by these bases. Vinograd and his co-workers (19) have pointed out that denatured DNA should form two bands in an alkaline CsCl gradient if there is an asymmetric distribution of G+T residues between complementary strands of DNA. They examined the DNA from several bacterial species and observed only a single band in an alkaline CsCl gradient. In this communication it is shown that there is a bias in G+T content between complementary strands of representative members of the *Enterobacteriaceae*. Our examination includes the DNA prepared from *Proteus mirabilis* (40% G+C), *Escherichia coli* (50% G+C), and *Serratia marcescens* (58% G+C; reference 9).

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MATERIALS AND METHODS

Bacterial strains. A *P. mirabilis* strain Pm15, which is lactose and galactose negative and requires nicotinic acid, tryptophan, leucine, and thymine for growth, was used in these studies. This strain was derived from *P. mirabilis* F67 which has been described previously (14) by successive *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis. The strains of *E. coli* K-12 W677 and *S. marcescens* SM6 S^r-11 which were used have been described previously (14).

Media. Penassay broth (Difco) was used. Bacterial growth was monitored by turbidity measurements at 650 nm.

Isolation of DNA. DNA was isolated by the procedure which has been described previously (12).

Lysis of bacteria at alkaline pH. To determine optimal conditions for lysing the bacterial cells at alkaline *p*H, preliminary experiments were carried out at room temperature in which washed *P. mirabilis* cells were resuspended in 1 ml of saline-ethylenediaminetetraacetic acid (SV: 0.1 M disodium EDTA, 0.1 M NaCl, adjusted to *p*H 8.0 with NaOH) at different cell densities. The addition of an equal volume of 0.4 M K_3PO_4 which had been titrated to *p*H 13 with KOH to the cell suspensions resulted in cell lysis. The lysate has a final *p*H of 12.5 ± 0.2. The rate of lysis was estimated from the decrease in absorbance with time at 650 nm by using a Gilford model 240 spectrophotometer. In these experiments the absorbance decreased to 15% of its original value in less than 2 min after addition of K_3PO_4 and then remained essentially constant for at least 1 hr. In the case of *P. mirabilis* cells, the extent of lysis appeared to be independent of culture age. With *E. coli* cells the extent of lysis shows a slight dependence on cell age, with stationary-phase cultures showing less clearing. To determine the release of DNA accompanying alkaline lysis of the cells, lysates were clarified by centrifugation and assayed by the Burton (3) procedure with deoxyadenosine used as a standard. When compared to the DNA content of the bacterial cells, these

measurements indicated that release of DNA after alkaline lysis of the cells was essentially quantitative. Although the extent of lysis of the cells as estimated from the absorbancy changes varied slightly with increasing cell density in the suspension (75% lysis at 1.2×10^9 cells/ml as compared to 50% at 40×10^9 cells/ml), the quantitative release of DNA was not dependent on the cell density. The extent of the absorbancy changes was enhanced by the addition of saturated CsCl to the lysate.

In experiments in which bacterial cells were lysed directly in an analytical ultracentrifuge cell, 0.07 ml of cell suspension containing 2.5×10^9 to 3.0×10^{10} bacteria/ml in SV, 0.07 ml of 0.4 M K_3PO_4 which had been titrated to *p*H 13 with KOH, and 0.56 ml of a saturated CsCl solution in water were added in sequence at room temperature to an assembled 12-mm ultracentrifuge cell by using a syringe. The resulting solution had a final *p*H of 12.5 ± 0.2 and an isopycnic density of 1.740 g/cm³. These values were measured after thoroughly mixing the contents of the ultracentrifuge cell after the completion of the ultracentrifuge run.

Denaturation of DNA. DNA was alkali denatured by mixing 0.05 ml of 0.4 M K_3PO_4 (*p*H 13) with 0.05 ml of DNA solution in SSC (0.15 M NaCl plus 0.015 M sodium citrate)/10. After standing at room temperature for 10 min, the solution was neutralized by adding 0.04 ml of 1.0 M potassium phosphate buffer, *p*H 5.5. In some experiments the DNA was thermally denatured by heating in a boiling-water bath for 2 min and rapidly cooling in an ice-water bath.

CsCl density gradient centrifugation. DNA samples were examined in a neutral CsCl gradient (*p*H 8.0) as described previously (12, 14). In alkaline CsCl gradient experiments, the centrifugation solution contained: 0.1 ml of DNA in SSC/10, 0.1 ml of 0.4 M K_3PO_4 at *p*H 13, and 0.8 ml of a stock-saturated CsCl solution in water. This solution had a *p*H of 12.5 ± 0.2 (reproducible instrument reading) and a density of 1.740 g/cm³. Buoyant densities were calculated as described by Schildkraut, Marmur, and Doty (17) using poly deoxy AT as a density standard (neutral gradient, ρ = 1.679 g/cm³; alkaline gradient, ρ = 1.739 g/cm³.

RESULTS

Asymmetry in G+T content between complementary strands of P. mirabilis DNA. When either native or heat-denatured *P.* mirabilis DNA is examined in a neutral CsCl gradient (*p*H 8.0), both types of DNA form unimodal bands as shown in Fig. 1 A and B, respectively. In similar experiments employing DNA which was denatured at alkaline *p*H and reneutralized prior to centrifugation, a minor band having the density of native *P. mirabilis* DNA was observed in the density profile (*data not shown*). This minor species presumably represents the naturally occurring cross-linked fraction of bacterial DNA which renatures spontaneously after removal of the DNA denaturing conditions (1, 13). When *P. mirabilis* DNA is examined in an alkaline CsCl gradient (*p*H 12.4), two DNA bands which differ

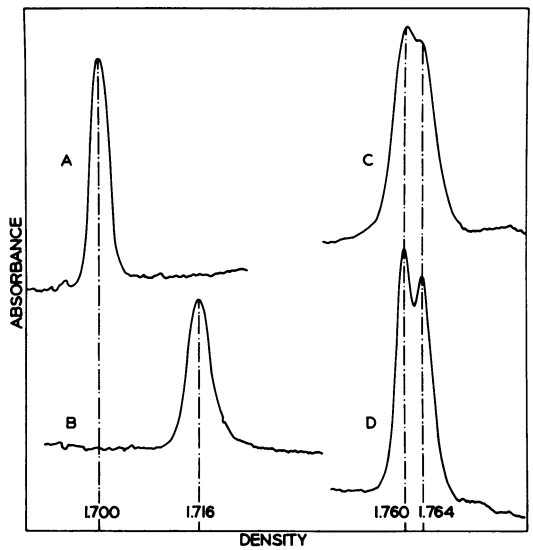

FIG. 1. *Density profiles of P. mirabilis DNA. (A) Native DNA in a neutral CsCl gradient (pH 8.0); (B) denatured DNA in a neutral CsCl gradient (pH 8.0); (C) isolated DNA in an alkaline CsCl gradient (pH 12.4); (D) DNA from bacterial cells which were lysed directly in an alkaline CsCl gradient (pH 12.4) in an analytical ultracentrifuge cell.*

in density by 0.004 g/cm³ are observed in the density profile (Fig. 1C). This is indicative that there is an asymmetry in the mole fraction of G+T residues between the complementary strands of *P. mirabilis* DNA.

An asymmetry in G+T content between complementary strands of chromosomal DNA from bacteria could occur in either of two ways as depicted schematically in Fig. 2. First, there could be a uniform asymmetry in G+T content between the complementary strands throughout the entire length of the chromosome (Fig. 2A). In this situation two bands of essentially the same density separation should be formed in an alkaline CsCl gradient when DNA samples of widely different molecular weight are examined. Second, G+T-rich segments may occur in different regions of both strands of the chromosome as illustrated in Fig. 2B. According to this view, the formation of the two bands in an alkaline CsCl gradient would be due to the fact that the molecular weight of our DNA preparations would approximate the size of the G+T-rich segments in the DNA. In this situation, examination of higher molecular weight DNA would tend to abolish the density differences between the complementary strands of the DNA. Since higher molecular weight DNA strands would contain segments both rich and poor in G+T, the overall G+T content of the two strands would be more nearly

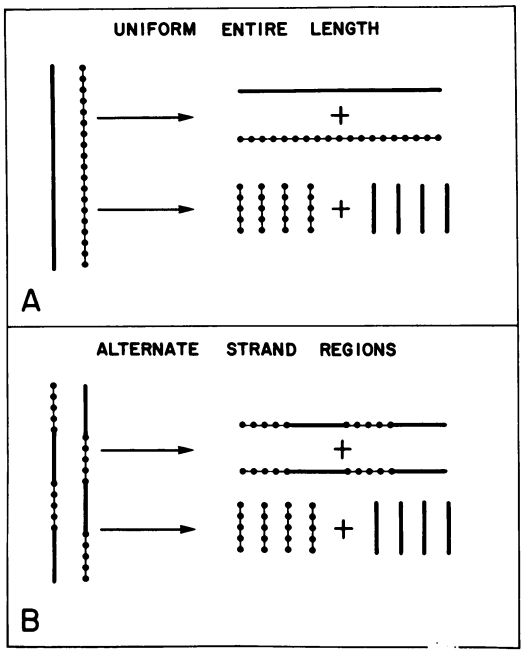

FIG. 2. *Schematic illustration of two possibilities for asymmetry in mole fraction of G+T between complementary strands of DNA.*

equivalent.

To distinguish between these two possibilities, *P. mirabilis* cells were lysed directly in an analytical ultracentrifuge cell in alkaline CsCl solution as described in Materials and Methods. McGrath and Williams (8) previously showed that extremely high-molecular-weight DNA can be prepared from cells which are lysed at alkaline *p*H under conditions which minimize handling of the DNA solution. In our experiments, the widths of the two DNA bands observed when cells were lysed directly in an alkaline CsCl gradient (Fig. 1D) were considerably narrower than when isolated DNA was examined under the same conditions (Fig. 1C), as would be expected for DNA strands of much higher molecular weight. However, lysis of the cells directly in the ultracentrifuge cell did not significantly change the observed density difference between the two bands observed in an alkaline CsCl gradient (Fig. 1D). These findings suggest that there is a relatively uniform asymmetry in G+T content between the complementary strands of *P. mirabilis* DNA throughout the entire length of the bacterial chromosome.

Asymmetry in G+T content between the complementary strands of E. coli and S. marcescens DNA. A set of density profiles of *E. coli* and *S. marcescens* DNA which were obtained in experiments similar to the ones just described for *P. mirabilis* DNA are assembled in Fig. 3 and 4, respectively. The results of these experiments are essentially the same as the previous ones except for the following two points. First, the denatured *E. coli* and *S. marcescens* DNA which was examined in a neutral CsCl gradient was prepared by alkali denaturation and reneutralization of the DNA. In these cases minor (cross-linked) bands

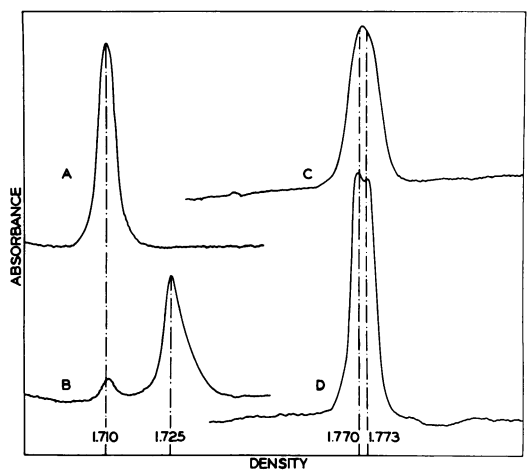

FIG. 3. *Density profiles of E. coli DNA. (A) Native DNA in a neutral CsCl gradient (pH 8.0); (B) denatured DNA in a neutral CsCl gradient (pH 8.0); (C) isolated DNA in an alkaline CsCl gradient (pH 12.4); (D) DNA from bacterial cells which were lysed directly in an alkaline CsCl gradient (pH 12.4) in an analytical ultracentrifuge cell.*

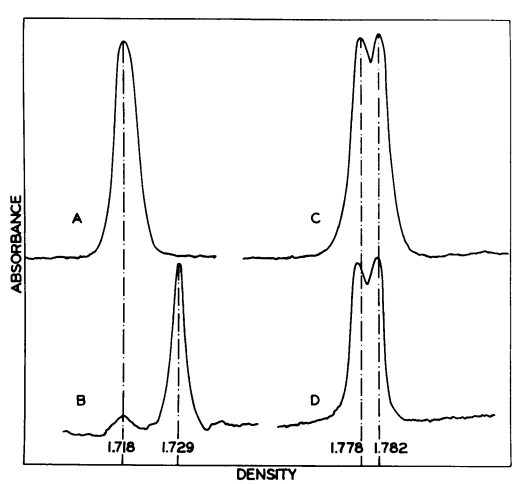

FIG. 4. *Density profiles of S. marcescens DNA. (A) Native DNA in a neutral CsCl gradient (pH 8.0); (B) denatured DNA in a neutral CsCl gradient (pH 8.0); (C) isolated DNA in an alkaline CsCl gradient (pH 12.4); (D) DNA from bacterial cells which were lysed directly in an alkaline CsCl gradient (pH 12.4) in an analytical ultracentrifuge cell.*

having the corresponding native DNA density were observed in the density profiles of the DNA (Fig. 3B and 4B, respectively). Second, isolated *E. coli* DNA did not form two resolvable bands in an alkaline CsCl gradient (Fig. 3C), whereas two bands were observed when *E. coli* cells were lysed in alkaline CsCl directly in the analytical ultracentrifuge cell (Fig. 3D). This difference is probably due to the difference in the molecular weight of the DNA in the two experiments. The DNA of the complementary strands forms very narrow bands when the cells are lysed directly in alkaline CsCl because the DNA has a very high molecular weight. As a result, resolution of the two bands is possible even though the density difference between the complementary strands is quite small. However, when isolated DNA was examined, the resolution of the two bands would be obscured due to the increased bandwidth of the lower molecular weight DNA.

DISCUSSION

Our examination of the chromosomal DNA prepared from three members of the family *Enterobacteriaceae* in an alkaline CsCl gradient revealed that there is a bias in the mole fraction of G+T residues between the complementary strands of the DNA. This asymmetry is most evident when DNA of very high molecular weight is examined. The density difference between the two DNA bands observed in an alkaline CsCl gradient does not appear to vary appreciably as a function of the molecular weight of the DNA. This suggests that there is a relatively uniform bias in G+T content between complementary strands throughout the entire lengths of the chromosomes of the three genera which were examined.

In a previous investigation, Vinograd and coworkers (19) had not observed that *E. coli* DNA formed two bands in an alkaline CsCl gradient. The difference between their results and our own would seem to be that the DNA in their experiments was of too low a molecular weight to resolve the two bands formed in an alkaline CsCl gradient.

There have been several examples reported of a difference in G+T content between the complementary strands of bacteriophage DNA (6), satellite DNA in eukaryotes (4, 5), and mitochondrial DNA (2). By using data from these investigations, it is possible to calculate that a difference in density of 0.001 g/cm^3 between complementary strands in an alkaline CsCl gradient corresponds to a difference of 0.003 in mole fraction G+T. Other experiments from this laboratory have shown that there is essentially no bias in thymine (and hence adenine) content between the complementary strands of *P. mirabilis* DNA (11; H. Kasamatsu, C. Hershberger, and R. Rownd, *manuscript in preparation*). Thus in this genus there must be a difference of approximately 0.012 in the mole fraction of guanine (and hence cytosine) between the complementary strands of the DNA. It would be of interest to determine whether such asymmetry in base composition between complementary strands is due to the clustering of specific DNA bases within the polynucleotide chains. In a number of instances, pyrimidine clusters have been implicated in the control of transcription by RNA polymerase (18).

Over the past few years several different methods for fractionating complementary strands of DNA have been introduced (6, 7, 15, 16). Chargaff and his collaborators (15) have developed a technique for separation of complementary strands which utilizes intermittent gradient elution from a column of methylated albumin kieselguhr. By employing chemical analysis of the base composition of complementary strands, they observed a difference in the mole fraction of G+T residues between the complementary strands of *E. coli* and *S. marcescens* DNA of 0.0105 and 0.0140, respectively. Although Chargaff indicated that these values were within the experimental error of the chemical measurements, his values compare quite favorably with our findings of differences in mole fraction G+T between the complementary strands of *E. coli* DNA and *S. marcescens* DNA of 0.009 and 0.012, respectively. Separation of complementary strands in an alkaline CsCl gradient may provide a more sensitive method of estimating asymmetry in G+T content between complementary strands of DNA than physical separation and subsequent chemical analysis.

ACKNOWLEDGMENTS

We thank John Anderegg for use of the Joyce-Loebl microdensitometer used in these studies.

This work was supported (in part) by Public Health Service research grant GM 14398 and by Research Career Development Award GM 19206 (to R.R.) from the National Institute of General Medical Sciences.

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