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Scission of *Escherichia coli* Deoxyribonucleic Acid in Alkali†

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ABSTRACT: DNA released from *Escherichia coli* spheroplasts exhibits an apparent decrease in molecular weight when centrifuged in alkaline sucrose gradients for long times (low rotor speeds) compared to shorter times (higher rotor speeds). Most of this decrease in molecular weight can be accounted for by alkali-catalyzed scission of DNA strands during centrifugation. At 0.3 N NaOH, *E. coli* DNA in crude lysates is cleaved at a rate of about one break/10^9 daltons per hr. λ closed duplex DNA rings are cleaved at a similar rate. Prolonged exposure of high molecular weight cellular DNAs to alkali will result in cleavage and an underestimation of single-strand molecular weights.

A common procedure for determination of the molecular weight of DNA single strands involves treatment of duplex DNA at alkaline pH. It has been known for a long time that, whereas RNA is rapidly hydrolyzed under these conditions, DNA appears to be quite stable. Nevertheless, there is evidence that alkali produces scissions in DNA, albeit at a slow rate. Purines are lost from the DNA backbone at alkaline pH (Greer and Zamenhof, 1962), and these sites are subsequently hydrolyzed (Shapiro and Chargaff, 1964). During experiments concerned with the effect of DNA synthesis inhibitors on *Escherichia coli* DNA molecular weight, we observed that DNA sedimented in alkaline sucrose gradients exhibited an apparently lower molecular weight when centrifuged for longer times. In this report we show that most of this effect results from alkaline cleavage of DNA strands. Rates of scission of DNA in alkali are high enough to be consequential in studies on the chromosomal DNA molecules of bacteria and eukaryotic cells which are 10^9 daltons and larger.

Experimental Procedure

Bacteria and Culture Conditions. The bacteria were derived from *E. coli* K12SH-28, a thymidine phosphorylase negative (Tpp$^-$) strain (Fangman, 1969): FA220 (Tpp$^-$, Arg$^-$, Lac$^-$, Tdr$^-$ (thymidine requiring)), FA210 (Tpp$^-$, λ$^+$ lysogen). All experiments were carried out with aerobic exponential phase cultures at 37°. For experiments with bacterial DNA, FA220 was grown in F buffer (Sadler and Novick, 1965) supplemented with 4 mg/ml of glucose, 2 mg/ml of decolorized Casamino Acids, and 2 μg/ml of [*methyl*-^{3}H]thymidine (50 μCi/ml) for at least three generations. Covalently closed λ DNA duplexes (form I) were produced by infecting strain FA210 with ^{3}H-labeled λ CI857 at a multiplicity of infection of 10 and incubating for 90 min (Fangman and Feiss, 1969).

Zone Sedimentation. On-gradient lysis of cells was carried out by a procedure similar to that of McGrath and Williams (1966). Approximately 3×10^8 cells (FA220 or λ-infected FA210) were suspended in 0.15 ml of 0.125 M sucrose–0.05 M EDTA–0.005 M Tris (pH 8). After addition of 0.04 ml of 1% lysozyme, samples were incubated 15 min at 37°. Spheroplasts were either layered directly onto sucrose gradients or lysed by addition of an equal volume of NaOH solution and incubated at 25°. Twenty-five microliters of material were layered onto 5–20% sucrose gradients (5 ml) containing either 0.3 N NaOH–0.7 M NaCl or 0.1 N NaOH–0.9 M NaCl. After 10 min at room temperature, the tubes were centrifuged in a Spinco SW50L rotor at 25°. With this rotor a speed of 10,000 rpm produces a centrifugal force of 8161*g* at the midpoint of the sample tube. Fractions collected from the bottom of the tubes were made 5% in trichloroacetic acid, and the precipitate washed on glass fiber filters with cold 5% trichloroacetic acid and 95% ethanol. Gradients contained at least 7000 cpm of [^{3}H]DNA.

[^{3}H]DNA released from λ virus particles by incubation with 1% sodium dodecyl sulfate for 30 min at 37° was employed as a sedimentation standard. Number-average molecule weight (M_n) of *E. coli* DNA was calculated as $M_n = 1/\Sigma(c_i/m_i)$, where c_i = fraction of total counts per minute in a gradient fraction and m_i = the molecular weight of DNA in that fraction (Fangman and Russel, 1971). Molecular weights were calculated from the equation of Studier (1965) for sedimentation in alkali. The number of breaks per 10^9 daltons of *E. coli* DNA was calculated as: $(1 \times 10^9$ daltons/observed $M_n) - 1$.

The fraction of ^{3}H-labeled λ DNA existing as form I was determined (Fangman and Feiss, 1969) by centrifuging samples through sucrose gradients containing 0.3 N NaOH–0.7 M NaCl at 32,000 rpm for 55 min (25°). The amount of unbroken form I

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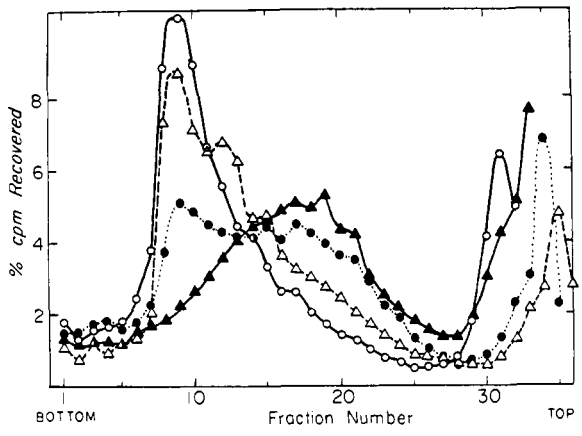

FIGURE 1: *E. coli* DNA released from spheroplasts layered on alkaline (0.3 N NaOH) sucrose sedimented at various rotor speeds. (○) 0.80 hr at 40,000 rpm; (△) 1.25 hr at 32,000 rpm; (●) 2.05 hr at 25,000 rpm; (▲) 12.8 hr at 10,000 rpm.

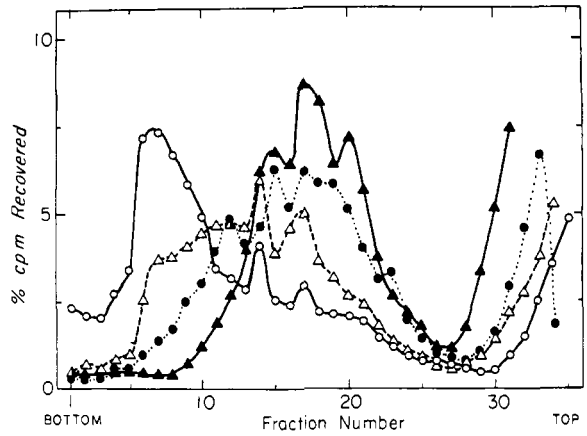

FIGURE 2: *E. coli* DNA sedimented through alkaline (0.3 N NaOH) sucrose after incubation with 0.3 N NaOH (25°) for various times. All centrifugations were at 32,000 rpm for 1.25 hr. Incubation times include the 1.25-hr centrifugation. (○) 1.25 hr; (△) 9.0 hr; (●) 13.25 hr; (▲) 19.25 hr.

molecules is taken as the percentage of radioactivity recovered in the fastest sedimenting peak. The time required to reduce the original amount of form I by a factor of e gives the times required for the population of molecules to sustain an average of one break per molecule.

Results

Figure 1 shows the sedimentation patterns of *E. coli* DNA released from spheroplasts and centrifuged through sucrose gradients containing 0.3 N NaOH at 10,000, 25,000, 32,000, and 40,000 rpm for times such that $(\text{rpm})^2 \times$ time remained constant. Below 32,000 rpm (longer than 1.25 hr) the pattern of DNA is shifted greatly toward lower sedimentation values. Above 32,000 rpm, the pattern is fairly constant and similar to that originally reported (McGrath and Williams, 1966). The apparent M_n drops from 1.3×10^8 daltons at 40,000 rpm (0.8 hr) to 0.62×10^8 daltons at 10,000 rpm (12.8 hr). If this difference is the result of cleavage of DNA molecules by alkali during centrifugation, the rate of scission would be approximately 1 break/10^9 daltons per hr.

To estimate the rate of scission of *E. coli* DNA in alkali more directly, spheroplasts were lysed by adding NaOH to 0.1 or 0.3 N, and incubated at 25°. At various times, material was transferred to alkaline (0.3 N NaOH) sucrose gradients and centrifuged at 32,000 rpm for 1.25 hr. Pipetting the lysed material had little effect on the DNA molecular weight since the sedimentation pattern obtained at "zero" time of incubation is very similar to that obtained when spheroplasts are layered directly onto the alkaline gradients. Sedimentation profiles for DNA treated with 0.3 N NaOH for various lengths of time are shown in Figure 2. The number of breaks for the two NaOH concentrations was calculated and plotted as a function of time in Figure 3. Rates of scission are given in Table I. With 0.3 N NaOH, the rate is approximately 0.7 break/10^9 daltons per hr. The rate with 0.1 N NaOH is about three times lower.

For comparison with the scission rate obtained with the *E. coli* DNA, the rate of conversion of λ covalently closed ring

TABLE I: Rates of DNA Breakage during Exposure to NaOH.[a]

DNA	NaOH (N)	Breaks/10^9 Daltons per hr
E. coli	0	0.112 ± 0.050[b]
	0.1	0.256 ± 0.057
	0.3	0.729 ± 0.113
λ form I	0	0.112 ± 0.218[b]
	0.1	0.221 ± 0.197[b]
	0.3	1.70 ± 0.234

[a] Rates and their standard errors were determined from least-squares linear estimates of the data in Figures 3 and 4. Values for λ form I are based on a λ DNA molecular weight of 3×10^7 daltons (Davidson and Szybalski, 1971). [b] These values are not significantly different from zero at the 5% confidence level.

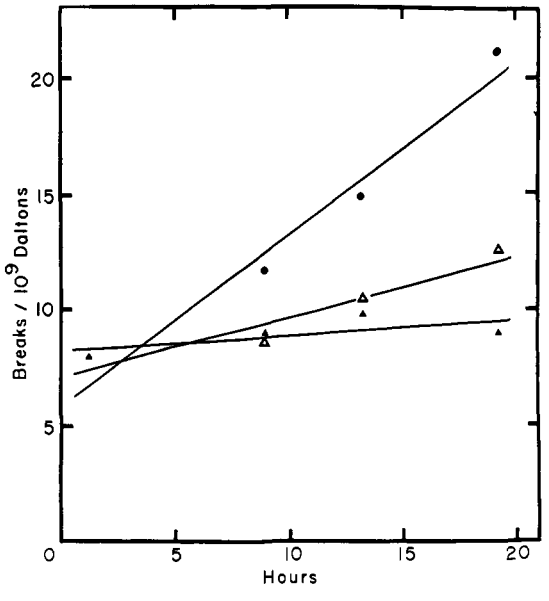

FIGURE 3: Occurrence of single-strand breaks in *E. coli* chromosomal DNA during incubation with alkali. M_n of the control DNA was 1.2×10^8 daltons, The 1.25-hr point was used in the determination of each of the three breakage rates. (▲) Control; (△) 0.1 N NaOH; (●) 0.3 N NaOH.

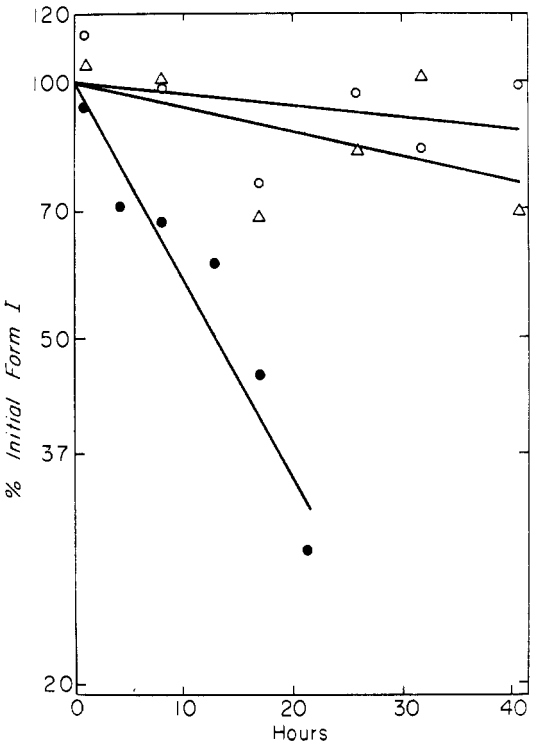

FIGURE 4: Decrease in the amount of form I λ DNA molecules after incubation with alkali for various times. (○) Control; (△) 0.1 N NaOH; (●) 0.3 N NaOH.

DNA molecules (form I) to molecules with one strand scission (form II) was measured. Spheroplasts of an *E. coli* λ lysogen infected with ^{3}H-labeled (DNA) λ viruses were lysed by adding NaOH to 0.1 or 0.3 N and incubated at 25°. The relative fraction of form I molecules is plotted *vs.* the times of incubation with NaOH in Figure 4, and the rates of scission calculated from these data are given in Table I. With 0.3 N NaOH, the rate is 0.051 break/λ form I per hr or 1.7 breaks/10^9 daltons per hr.

Discussion

Exposure to alkali results in cleavage of both λ and *E. coli* DNA. The rates of alkali-induced scission determined for the two DNAs differ by about a factor of two. This difference may mean that the empirical equation developed by Studier (1965) does not precisely apply to our conditions of sedimentation of the bacterial DNA. It is likely, however, that the difference results from systematic errors in the calculation of the M_n of *E. coli* DNA which is very sensitive to changes in the proportion of material at the lower sedimentation values. Another possibility is that the faster rate of scission observed with λ DNA form I reflects an inherently greater lability of one bond in the λ DNA molecule, since the technique measures only the first bond cleaved in each form I molecule.

The experimentally determined rates of scission for the *E. coli* DNA (Table I) presumably reflect the average lability of each base (or at least each purine) in the DNA. An alternative possibility is that there are a smaller number of labile sites in the DNA molecule (*e.g.*, Bendich *et al.*, 1964), such as would exist if some of the RNA primers of nascent DNA fragments (Sugino *et al.*, 1972) were not excised before ligation.

The rate of alkali-catalyzed scissions in *E. coli* DNA is great enough to account for most of the decrease in apparent molecule weight observed at long centrifugation times. Analysis of the single-strand molecular weight of large DNA molecules such as those found in eukaryotic chromosomes will need to be carried out under conditions which avoid prolonged exposure to alkali.

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