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CORRESPONDENCE

The Yield and Repair of X-Ray-induced Single-Strand Breaks in the DNA of *Escherichia coli* K-12 Cells

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The yield of single-strand breaks present in chromosomal DNA of *Escherichia coli* cells after aerobic X irradiation was determined using alkaline sucrose gradient techniques. Experimental conditions were used that avoided a centrifugation speed dependence effect on DNA sedimentation, and allowed either minimal or complete enzymatic repair to occur. The results confirm the qualitative conclusions made in earlier reports from this laboratory, but indicate that the extent of DNA strand breakage was previously underestimated by factors of 3.4 to 7.6, depending on the strain and repair conditions used. Technical and interpretive difficulties in the measurement of DNA single-strand breakage using alkaline sucrose gradient techniques are discussed briefly. In the present experiments, the initial yield of DNA single-strand breaks was found to be 32.4/genome krad^{-1} (9.0 eV/break).

INTRODUCTION

There are several problems with the use of the alkaline sucrose gradient technique developed by McGrath and Williams (*1*) for measuring the extent of DNA single-strand breakage in *Escherichia coli* cells. For example, it has been found that large DNA molecules are subject to a centrifugation speed effect such that the apparent sedimentation rate of the DNA decreases as the speed of centrifugation is increased above a certain critical level [e.g., (*2, 3*)]. Therefore, samples containing large molecular weight DNA must be centrifuged at slow speeds to avoid this effect. In addition, nonrandom populations of DNA molecules are obtained from cells with only small amounts of DNA strand breakage (*4, 5*), thus invalidating, in such cases, the use of weight average molecular weight calculations to estimate a number average molecular weight (*6*). Both of these factors contributed to miscalculations of DNA strand breakage values in earlier reports (*7–13*). An additional error in these earlier reports arose from an overestimate of the molecular weight of DNA samples from unirradiated cells (*10*).

In this report we have reevaluated some of our earlier data [from Ref. (*7*, Fig. 4)] and have added additional data in an effort to obtain a more accurate

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TABLE I

Strain and extent of repair	Single-strand breaks/10^8 krad^{-1} (from Fig. 1 or Ref.)	Single-strand breaks per E. coli genome (2.8×10^9)/krad		Ratio A/B[b]	eV/Break[c]
		A. Current data (Fig. 1)	B. Earlier data (Ref.)		
1. *polA*; minimal repair	1.16 ± 0.05	32.4 ± 1.3	4.26 (*8, 9, 11, 12*)	7.6	9.0 ± 0.3
	(0.97 ± 0.17)[a]	(27.2 ± 4.8)[a]	—	—	(10.7 ± 1.6)[a]
2. *resA*; minimal repair (200 kVp X rays)	0.72 (*18*)	—	—	—	14
3. *uvrA*; minimal repair (4 MeV electrons)	0.83–0.89 (*19*)	—	—	—	12
4. *polA*; after repair in buffer (Type II)	0.213 ± 0.010	5.97 ± 0.28	1.08 (*7*)	5.5	—
5. Wild-type; after repair in buffer (Type II)	0.061 ± 0.004	1.70 ± 0.1	0.38 (*7*)	4.5	—
			0.46 (*9, 12*)	3.7	—
6. Wild-type; after repair in growth medium (Types II and III)	0.057 ± 0.004	1.58 ± 0.1	0.46 (*9, 12*)	3.4	—
7. Extent of Type III repair in wild-type cells	—	7.6 (at 10 krad)	3 to 4 (*7, 9, 12*)	2.2	—

[a] ^{137}Cs γ irradiation (*17*). All other values are for 50 kVp X irradiation taken from Fig. 1, except those in rows 2 and 3 where the radiation quality is specified.

[b] The number of DNA strand breaks per half-genome in earlier reports from this laboratory (*7–13*) was calculated using the formula $6[(D_1/D_2)^{2.63} - 1]$ [Ref. (*10*)], where D_1 and D_2 are the first moments of the control and irradiated DNA profiles, respectively. There are several sources of error in this formulation: (i) According to the present results, the value 6 should be ~14. This is the ratio of the size of the *E. coli* half-genome (1.4×10^9) to that of the control sample (~1×10^8 from Fig. 1). Thus, a ~2.3-fold underestimate of the yield of strand breaks in the earlier calculations would result from this difference. (ii) The relationship $(D_1/D_2)^{2.63}$ is equivalent to the ratio of the weight average molecular weights. The nonrandomness of DNA samples from unirradiated cells invalidates the use of weight average molecular weights in strand break calculations (*6*); the number average molecular weight must be used. Since the ratio $M_w:M_n$ is ~2 for randomly broken DNA samples and ~1.4 for unirradiated samples (unpublished results), this could introduce a ~1.4-fold underestimate of DNA strand breakage if $(D_1/D_2)^{2.63} \gg 1$. (iii) The centrifugation speed effect would also tend to decrease $(D_1)^{2.63}$. With a Beckman SW50.1 rotor at 30,000 rpm, the conditions used in earlier reports (*7–13*), the reduction in apparent M_n relative to that observed at 20,000 rpm is ~1.3 fold, for samples with $M_n \sim 1 \times 10^8$ (unpublished results). (iv) The overall error effect from these three components is ~4.2-fold, a value that is in fair agreement with the experimentally determined differences between the earlier strand breakage data and the current results.

[c] The energy requirement per single-strand break (eV/break) was calculated using the current data, according to the relationship $(6.25 \times 10^{13})(X_2 - X_1)/(M_{n1}/M_{n2} - 1)(6.03 \times 10^{23}/M_{n1})$ = eV/single-strand break, where M_{n1} and M_{n2} are the number average molecular weights of DNA from cells receiving a dose of ionizing radiation, X_1 or X_2 (in rads), respectively.

estimate of the extent of DNA strand breakage in *E. coli* K-12 cells after ionizing irradiation.

MATERIALS AND METHODS

The characteristics of the strains used, the media and growth conditions, procedures for X irradiation, alkaline sucrose gradient techniques, and the method of cell lysis have been described in detail (*7*). Under these conditions the DNA from unirradiated cells had a sedimentation rate that was independent of the rotor speed up to approximately 21,000 rpm with a Beckman SW50.1 rotor. At speeds greater than this, the apparent S value of the DNA decreased. For the experiments reported here, centrifugation speeds were used that avoided this effect. The centrifugation conditions used ranged from 21,000 rpm for ~4 hr, for samples from unirradiated cells ($M_n \sim 1 \times 10^8$), to 35,000 rpm for various times, for DNA samples of low molecular weight ($M_n < 2 \times 10^7$).

The average molecular weight, M_n, was calculated directly from the gradient profiles, using phage T2 DNA labeled with ^{14}C-thymine as a molecular weight marker, according to the relationship

$$M_n = \frac{1}{(\sum f_i/d_i^{2.63} \text{ for } E.\ coli \text{ DNA})} \times \frac{55 \times 10^6}{(d^{2.63} \text{ for phage T2 DNA})},$$

where f_i is the fractional amount of recovered radioactivity, and d_i is the average distance sedimented by the ith fraction. The value 55×10^6 was used as the single-strand molecular weight for phage T2 DNA (*14*). The determination of the exponent value, 2.63, has been described (*2, 14, 15*). In calculating the value ($\Sigma f_i/d_i^{2.63}$ for *E. coli* DNA) only the fractions under the main peak area were used. For the phage T2 DNA calculation, the distance sedimented at the peak fraction was used.

RESULTS

The extent of DNA strand breakage was determined in *E. coli* K-12 wild-type and *polA* strains under conditions that allowed (i) little or no enzymatic repair to occur, (ii) completion of repair processes that can occur in buffer [Type II repair (*12*)], or (iii) completion of repair processes that can occur in growth medium [Types II and III repair (*12*)]. The results are shown in Fig. 1. In order of decreasing slope, the lines fitted to the data indicate the extent of DNA strand breakage immediately after aerobic X irradiation of *polA* cells, after Type II repair in *polA* cells, after Type II repair in wild-type cells, and after Types II and III repair in wild-type cells.

Table I shows the rate of strand breakage and the average energy deposition per break, as calculated from the data in Fig. 1. The values given in earlier reports are listed for comparison. The extent of Type III repair was calculated as the difference between the lines fitted to the data for the wild-type cells incubated in buffer and minimal medium at any given dose (Fig. 1). At 10 krad the difference between the two lines is equal to 7.6 single-strand breaks per genome.

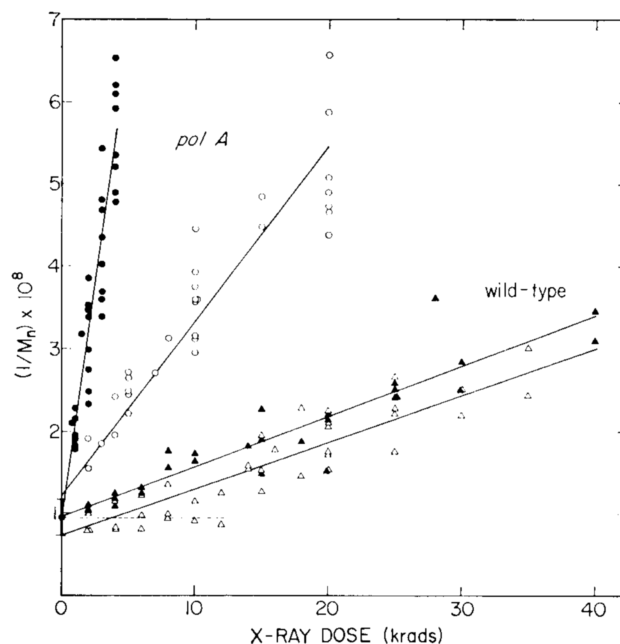

FIG. 1. DNA single-strand breakage after 50 kVp X irradiation. The number average molecular weight values were obtained by direct calculations from DNA profiles from alkaline sucrose gradients. Strains and conditions were: DY100 *polA1;* immediate lysis after irradiation at 0°C (●), 80 min incubation in DTM buffer (minimal medium without organic components) at 37°C after irradiation at room temperature (○). DY98 wild-type; 80 min incubation at 37°C in buffer (▲) or minimal medium (△) following irradiation at room temperature. The samples were aerated vigorously for 2–3 min prior to and during X irradiation. The lines were fitted to the data by linear regression analysis and have slopes, in order of decreasing magnitude, of 1.16, 0.213, 0.061, 0.057 DNA single-strand breaks/10^8 krad^{-1}. For unirradiated cells the mean $10^8/M_n$ value and its standard deviation are 0.94 ± 0.22. The dashed line is an extension of the $10^8/M_n$ value for unirradiated cells.

DISCUSSION

Measurements of the initial yield of radiation-induced DNA single-strand breaks in bacterial cells have shown a great deal of variability, probably because of the use of irradiation and cell lysis conditions that allowed repair to occur [e.g., see summaries in (*8*, *16*)]. The present data (Table I) were obtained under conditions allowing minimal repair and indicate that ~9 eV is required per DNA single-strand break in *E. coli* chromosomal DNA after aerobic X irradiation. The energy requirement for single-strand breakage after ^{137}Cs γ irradiation (*17*) was slightly greater, 10.7 eV, but the difference is not statistically significant. These values compare favorably with the lowest previously published values for *E. coli* chromosomal DNA breakage; 14 eV per break reported by Kato (*18*), and 12 to 16 eV per break reported by Johansen *et al.* (*19*). Thus, the deposition of 9 to 16 eV appears to be required to produce one single-strand break in *E. coli* chromosomal DNA by aerobic X irradiation.

Two problems plaguing the determination of molecular weights of large DNA

samples using sucrose gradient techniques are (i) a centrifugation speed effect that reduces the apparent sedimentation rate of large molecules (*2*, *3*), and (ii) the nonrandom characteristics of the DNA pieces obtained from unirradiated cells (*4–6*). The DNA strand-break calculations in earlier reports from this laboratory (*7–13*) are incorrect by factors of ~4 for wild-type cells and ~6.5 for *polA* cells, partly because of these technical problems (Table I). These earlier errors also resulted in a 2.2-fold underestimate of the extent of growth-medium-dependent (Type III) repair (Table I).

The present results indicate that incubation in buffer (to allow the completion of Type II repair) does not result in the complete repair of DNA strand breaks at low doses in either *polA* or wild-type cells. However, incubation in growth medium (to allow the completion of both Types II and III repair) results in the repair of most strand breaks in wild-type cells after low doses (0.5 krad) of X radiation, although the sensitivity of the method is such that a small number of breaks might not be detected. In contrast, the earlier reports (*7*, *9*, *12*) showed complete repair of DNA strand breaks after X-ray doses up to ~4 krad in buffer, and ~10 krad in growth medium, probably because of a centrifugation speed effect that would mask low levels of DNA strand breakage and give the impression of complete repair.

The DNA strand breaks that should correlate most closely with cell survival are those that remain unrepaired after all repair activity has ended. These breaks would arise from initial radiochemical events and also from abortive excision repair of base damage (*20–23*). Our results for the wild-type strain indicate that approximately 1.58 DNA single-strand breaks remain per chromosome of 2.8×10^9 daltons/krad after incubation in growth medium to allow the completion of Types II and III repair (Table I). Since the D_0 value for the wild-type strain is ~2.3 krad (*7*), an average of ~3.6 single-strand breaks remain unrepaired per genome per lethal event. Thus, more DNA strand breaks remain than would be required to account for cell killing if each unrepaired single-strand break constituted a lethal event.

There are several factors that complicate attempts to quantify the correlation between unrepaired strand breaks and cell survival: (i) A portion of the unrepaired single-strand breaks arises from double-strand breaks, each of which would yield two single-strand breaks. The total yield of DNA strand breaks can be calculated if both the single- and double-strand break yields are known (*17*). (ii) Part of the single-strand breaks result from nonbreak lesions that are sensitive to alkaline conditions (alkali-labile sites). Published data suggest that such lesions may be lethal (*24*) and nonrepairable (*25*). (iii) DNA degradation in irradiated cells could lead to either an over- or an underestimation of DNA strand breakage. It is difficult to make a suitable correction for this problem. (iv) Additional repair of strand breaks could occur during the time interval between the end of repair measurements and the assay for cell survival. (v) Lesions that do not result in DNA strand breaks (e.g., base changes) are likely to contribute to cell killing. (vi) Cells that have replicated a portion of their genome would have areas of DNA redundancy (the replicated regions), and should not be subject to single-hit inactivation kinetics.

Thus, the measurement of DNA strand breakage and attempts to correlate such data with cell survival are subject to several technical and interpretive difficulties. The technical problems of speed dependence and nonrandomness of DNA samples can generally be overcome. The interpretive problems in correlating DNA strand breakage and cell survival can be evaluated in some instances, but not in others. It is apparent that attempts to correlate DNA damage with cell killing quantitatively, although useful, must be judged carefully.

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