RELATIVE RATES OF REPAIR OF SINGLE-STRAND BREAKS AND POSTIRRADIATION DNA DEGRADATION IN NORMAL AND INDUCED CELLS OF *ESCHERICHIA COLI*

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ABSTRACT Labeled DNA from irradiated *Excherichia coli* cells has been studied on an alkaline sucrose gradient without acid precipitation of the DNA. This enables the observation of both DNA repair and DNA degradation. The use of a predose of ultraviolet light (UV) causes induction of an inhibitor of postirradiation DNA degradation in *lex*$^+$ strains. The effect of this induction on both the repair of single-strand breaks and DNA degradation has been followed in strains WU3610 (*uvr*$^+$) and WU3610-89 (*uvr*$^-$). The repair process is more rapid than the degradation, and when degradation is inhibited more repair is apparent. Cells that are *lex*$^-$ (B_{s-1} and AB2474) cannot be induced for inhibition of degradation. Nevertheless, by observation at short times repair can be seen clearly. This repaired DNA is degraded, suggesting that the signal for DNA degradation is not a single-strand break.

INTRODUCTION

Cellular response to radiation involves mechanisms that increase survival of the lethal and mutagenic effects of radiation. This ability of cells to survive has been attributed, in part, to enzymatic repair processes that act on the damaged DNA. Using a strain resistant to radiation, *Escherichia coli* B/r, and one sensitive to radiation, B_{s-1}, McGrath and Williams (1966) showed that X-ray damage to bacterial DNA could be repaired. By using alkaline sucrose gradients for analysis of radiation-induced single-strand breaks, they showed that by 40 min after irradiation the DNA of B/r had been almost completely repaired, whereas the DNA of B_{s-1} apparently had not. Much work followed in this area, and three classes of strand-break repair characterized by widely different kinetics were seen by Town et al. (1972). A slow repair, which takes 40–60 min to complete, was called type III repair; a faster repair, which requires the *polA* gene product and occurs within 2–5 min after irradiation, was called type II repair; and an ultrafast repair, which mends single-strand breaks within 1 min at 0°C, was called type I repair. The extensive amount of DNA degradation produced after irradiation in B_{s-1} led Horan et al. (1972) to suggest that B_{s-1}

could repair single-strand breaks, and perhaps the DNA degradation somehow obscures it.

However, the cellular response to radiation is much more complex than simple strand-joining repair processes. More recently, it has become established that some cells respond to agents that damage their DNA, such as ultraviolet (UV), and subsequently express what is believed to be a coordinately regulated group of functions. Strains of *E. coli* that are *recA*$^+$ and *lex*$^+$ show several UV-inducible functions, including induction of prophage λ, reactivation of irradiated phage (Weigle, 1953), induction of filament formation (Witkin, 1967), inhibition of postirradiation DNA degradation (Pollard and Randall, 1973), and an error-prone DNA repair activity related to mutation (Witkin and George, 1973). These are sometimes referred to as "s.o.s." functions (Witkin, 1976); we prefer the term "induced repair."

The work presented in this paper concerns induced inhibition of postirradiation DNA degradation and repair of single-strand breaks caused by ionizing radiation. Early observations by Miletić et al. (1961, 1964) suggested the presence of the degradation inhibitor in irradiated cells. The work of Grady and Pollard (1968) confirmed these findings and suggested that radiation acts to cause the initiation of DNA degradation and to induce a factor controlling the amount of degradation as well. Pollard and Randall (1973), using preliminary UV treatment, together with a subsequent prevention of transcription with rifampin, demonstrated the time-course of its induction and some of the strains in which it was found. Marsden et al. (1974) showed that the induction of the inhibitor of degradation could not be found in cells that were *recA*$^-$ or *lex*$^-$, suggesting that these genes were involved with the inhibition. The process of induction was shown by Tolun et al. (1974) to increase the amount of repair of radiation-produced single-strand breaks. The reason for this could be the reduction in DNA degradation due to induction or to some additional repair process.

We were led to investigate the nature of repair and degradation in UV-induced and uninduced cells, using an alkaline sucrose gradient technique modified for this purpose. Our findings are (*a*) that the repair and degradation processes proceed at different rates, repair being faster, (*b*) that upon induction by UV there is more repair, best correlated with the reduction of degradation, (*c*) that repair does occur in *lex*$^-$ cells such as B_{s-1}, and (*d*) that repaired DNA can be, and often is, degraded.

MATERIALS AND METHODS

Bacterial Strains

The *E. coli* strains WU3610, a B/r strain, and its *uvr*$^-$ derivative, WU3610-89, were obtained from Dr. E. M. Witkin via Dr. Stanley Person, Pennsylvania State University. *E. coli* strains B_{s-1} (*uvr*$^-$ *lex*$^-$) and AB2474 (*uvr*$^-$ *lex*$^-$), a K12 strain, were obtained from Dr. B. S. Bachmann of the *E. coli* Genetic Stock Center, Yale University Medical School, New Haven, Conn.

Growth Conditions

Cells were grown with aeration on Roberts' C-minimal medium (2 g NH_4Cl, 6 g Na_2HPO_4, 3 g KH_2PO_4, 3 g NaCl, 124 mg $MgCl_2 \cdot 6H_2O$, 80 mg Na_2SO_4/liter with 5 g glucose/liter and

a supplement of 2 g/liter of casamino acids. This medium is referred to as "casaC." Thiamine was added at 20 μg/ml final concentration for the AB2474 strain. At 37°C, doubling time was about 30 min.

Irradiation Methods

The UV source was a G15T8 germicidal lamp (GTE Sylvania Lighting Products, Hillsboro, N.H.) wrapped with insulation tape to diminish the yield when necessary. Two such bulbs were used, giving dose rates of 0.95 and 0.05 J/m^2 per s, as measured by a Jagger meter (Jagger, 1961), and calibrated against a Bureau of Standards standard lamp by Dr. R. A. Deering of our laboratories.

The ionizing radiation was delivered by a ^{60}Co Gammacell 200 (Atomic Energy of Canada, Ltd., Ottawa, Ontario) at a rate of 22.8 krad/min as determined by ferrous sulfate dosimetry. The cells were chilled on ice before irradiation, and were equilibrated with and bubbled with air during irradiation.

Procedure

Cultures were grown to a concentration of about 2 × 10^8 cells/ml in casaC medium containing deoxyadenosine at a final concentration of 0.25 mg/ml, and [^{3}H]thymidine at a final concentration of 5 μCi/ml. The cells were washed twice by repeated centrifugation in a Sorvall 55-1 rotor (Ivan Sorvall, Inc., Norwalk, Conn.) at room temperature and resuspended in growth medium.

For cells undergoing inducing treatment, the required UV dose, varied to fit the strain, was given. Previous studies (Pollard and Randall, 1973) show that for UV induction of the induced inhibitor there is a maximum at about 15 J/m^2 for wild-type strains and 1.5–2 J/m^2 for *uvr*$^-$ strains. After the UV treatment, the cells were bubbled with air at 37°C for 40 min to allow for synthesis of all induced proteins. These cells were then given rifampin at 50 μg/ml final concentration to prevent induction of any repair system by the gamma rays, incubated with bubbling for 10 min at 37°C, chilled on ice, and irradiated. For cells in which no UV-inducing treatment was given, rifampin was given immediately, and the procedure which followed was the same as described above. Cells that were *lex*$^-$ were given 12.1 krad of ^{60}Co gamma rays, and those not *lex*$^-$ were given 19 krad.

After irradiation the cells were immediately incubated with aeration at 37°C to allow for repair of the damage due to ionizing radiation. Samples from these cells were taken ranging from 0–45 min and iced. A small aliquot, 0.1 ml, of each sample was then layered and lysed on top of an alkaline sucrose gradient.

Alkaline Sucrose Gradient Technique

The alkaline sucrose gradients (5–18% sucrose, 0.9 M NaCl, 0.10–0.15 M NaOH, 0.003 M EDTA) were prepared at room temperature and layered gently with 0.15 ml lysing solution 0.1% sarcosyl (*N*-lauroyl sarcosine, Sigma Chemical Co., St. Louis, Mo.) 0.01 M EDTA, 0.5 M NaOH). Then 0.1 ml of the sample was layered onto the gradient, allowed to stand for 30 min for cell lysis, and then centrifuged at 30,000 rpm for 75 min at 20°C using a SW50.1 rotor in either a Beckman L3-50 or L2-65B ultracentrifuge (Beckman Instruments Inc., Palo Alto, Calif.).

The gradients were collected 10 drops at a time into 30 small (5-ml) glass vials. 0.25 ml of 3 M HCl and 3 ml of Scintiverse (Fisher Scientific, Fairlawn, N.J.) scintillation cocktail was added to each fraction which was then counted for 5 min in a Beckman LS-230 liquid scintillation counter (Beckman Instruments). To include a measure of the amount of DNA degraded, the total activity in each fraction was determined, rather than the acid-insoluble activity only,

as was done previously (McGrath and Williams, 1966). This procedure is in line with the work of Tolun et al. (1974).

RESULTS AND DISCUSSION

Fig. 1 shows typical results obtained with strain WU3610 (*uvr*$^+$) with no inducing treatment, but treated only with rifampin for 10 min before the administration of 19 krad of gamma radiation. The purpose of the rifampin treatment is to prevent induction of any repair system by the gamma radiation itself. Thus, induced inhibition of DNA degradation by the gamma rays cannot occur and the degraded DNA can be observed as fragmented DNA at the top of the gradient. The higher molecular weight (mol wt) DNA appears in the lower fractions. Under our conditions no reliable figures for molecular weight can be deduced because, as Zimm (1974) has theoretically derived, and Hutchinson and Krasin (1977) have experimentally confirmed, the viscous forces acting on random coil DNA so modify the sedimentation of DNA that there is a maximum rate. In our experiments we are concerned only to show whether or not radiation-damaged DNA increases in size under various conditions of treatment and to estimate the amount of DNA so repaired. Thus molecular weight figures are given

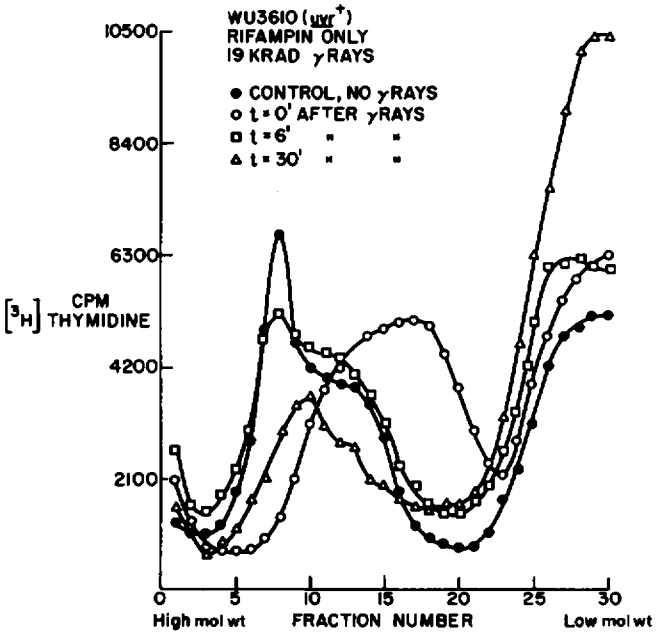

FIGURE 1 Alkaline sucrose gradient analysis of the labeled DNA of cells of WU3610 (*uvr*$^+$) which have been given rifampin and incubated for 10 min before receiving 19 krad of gamma radiation. Unirradiated cells show a characteristic double peak at high molecular weight (mol wt). Irradiation with no incubation yields fragmented DNA at a much lower molecular weight. Incubation for 6 min considerably restores the size of the DNA, but continuing the incubation to 30 min produces little further repair and considerable degradation, seen in the upsweep at the lower molecular weight end.

only to enable comparison with other similarly performed work. The peak at fraction 7 has a mol wt of approximately 4.5×10^8 daltons, as calculated following Studier (1965). We have found that the rifampin treatment gives somewhat smaller segments of DNA.

The broad peak in the zero-incubation-time case has a maximum at approximately 10^8 daltons. After 6 min of incubation, the distribution of the DNA is nearly like that of the control except that the peak is a little lower. Thus there has been considerable repair in only 6 min of incubation. After 30 min the distribution is still that of relatively large DNA but the amount is considerably less. Looking at the fractions containing highly fragmented DNA, which the work of Tolun et al. (1974) showed to be largely soluble in 5% trichloroacetic acid, we see a marked increase during the interval from 6 to 30 min. This, in the uninduced cells, is the product of the DNA degradation machinery, at least in part Exonuclease V, as suggested by the lack of DNA degradation in *recBC* strains.

In Fig. 2 we show the results obtained from the same strain, but previously treated with 17 J/m^2 UV-inducing dose. Rapid repair is again seen, but there is now less degradation so that the amount of the repaired DNA seen at 30 min of incubation is about the same as that at 10 min. This decrease in the amount of degradation we attribute to the induced inhibition of degradation, as described earlier.

We found similar data for strain WU3610-89 (*uvr*$^-$). In this instance the inducing

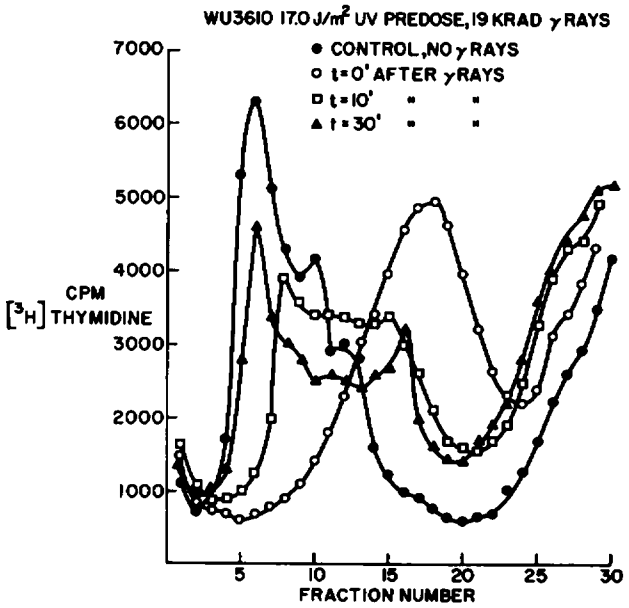

FIGURE 2 Observations similar to Fig. 1, except that a pretreatment with 17 J/m^2 of UV was given, followed by a 40-min incubation to allow protein synthesis before rifampin treatment and exposure to gamma rays. The amount of repair is considerable and is greatest after the longer incubation. At the same time there is very much less DNA degradation.

doses needed to produce maximum inhibition have been shown to be less than in the *uvr*$^+$ case, and the time to reach maximum inhibition is also different. The dose we used proved to be a little lower than that required to give maximum inhibition. The findings are summarized in Table I.

Inasmuch as we found the repair process to be nearly complete in 6 min, while degradation is not complete in 40 min, it is clear that repair proceeds more rapidly than does degradation. Accordingly, we decided to investigate whether *lex*$^-$ strains of *E. coli*, which have been previously reported not to show repair (McGrath and Williams, 1966), actually do show repair, but repair that is masked at longer incubation times, such as were previously used, by degradation.

In Figs. 3 and 4 we show data taken for strain AB2474 (*lex*$^-$). Fig. 3 shows the case where no UV was given and Fig. 4 that for a predose of 1.5 J/m^2 UV. It can be seen

TABLE I

APPROXIMATE ESTIMATES OF REPAIRED AND DEGRADED FRACTIONS

Strain	UV treatment	Incubation	Fraction repaired	Fraction degraded by gamma rays
		(*min*)		
WU3610 (*uvr*$^+$)	None	0	—	0.03
		6	0.8	0.10
		30	0.3	0.4
	17 J/m^2	0	—	0.05
		10	0.3	0.15
		30	0.6	0.15
WU3610-89 (*uvr*$^-$)	None	0	—	0.03
		10	0.5	0.06
		40	0.1	0.5
	1.5 J/m^2	0	—	0.03
		10	0.5	0.12
		40	0.3	0.35
B_{s-1} (*lex*$^-$, *uvr*$^-$)	None	0	—	0.02
		10	0.5	0.15
		40	0.15	0.65
	1.5 J/m^2	0	—	0.03
		10	0.4	0.15
		40	0.10	0.70
AB2474 (*lex*$^-$, *uvr*$^-$)	None	0	—	0.00
		10	0.6	0.20
		40	0.25	0.65
	1.5 J/m^2	0	—	0.05
		10	0.40	0.20
		40	0.15	0.55

The approximations of the fractions are made by estimating the total counts per minute in the fractions associated with the unirradiated peak and treating these as repaired. The proportion of the zero-time irradiated peak that moves into this association is then the fraction repaired. The fraction degraded is found similarly by treating the low molecular weight upswing as degraded DNA and estimating the counts per minute in that region. Again, the proportion of the zero-time irradiated peak that moves into this zone is called the fraction degraded. At best they are estimates, but they do permit the consideration of more data which cannot be shown because of space limitations.

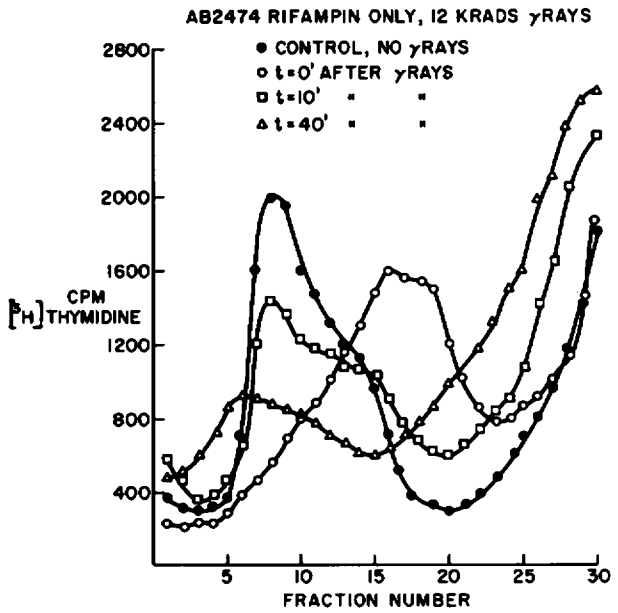

FIGURE 3 Data similar to those of Fig. 1 on the K12 strain AB2474, which is *uvr*$^-$ *lex*$^-$. There is considerable repair at short incubation times, but a reduction of the repaired DNA as degradation proceeds.

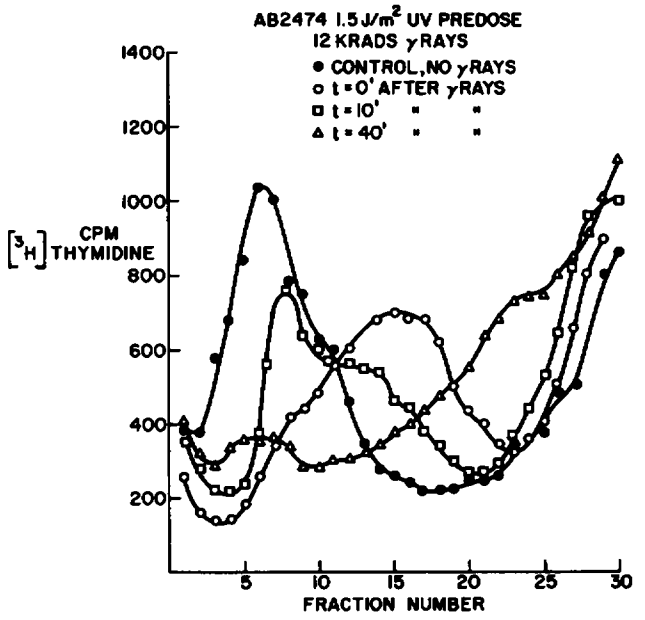

FIGURE 4 The effect of pretreatment with 1.5 J/m^2 of UV on AB2474. There is again no marked effect on either the repair or the degradation. Repair is slightly less than in the non-UV case, and the degraded fragments are perhaps larger.

that by 10 min of incubation, when DNA degradation is just beginning to be appreciable, considerable repair is apparent. At 40 min, degradation has proceeded, and it is clear that the repaired DNA is also degraded. Fig. 4 shows that the predose has been virtually without effect, diminishing, if anything, the amount of repair. This is in keeping with the *lex*$^-$ character of the cells.

We performed the same kind of experiments on strain B_{s-1}(*lex*$^-$, *uvr*$^-$) with similar results. Table I shows a summary of the appreciable amounts of repaired and degraded fractions for the four strains we examined. We can comment that when degradation is inhibited effectively (from separate observations of solubilization of DNA we estimate 80% inhibition for 17 J/m^2 for strain WU3610), the fraction repaired increases more slowly. Because there is about double the amount of DNA to be repaired, this suggests that a finite number of repair enzymes are active and that they have some limitation on rate. In the *uvr*$^-$ case (where we estimate 60% inhibition of DNA degradation for 1.5 J/m^2 UV), the amount of DNA available for repair is not increased so much and the inhibition is not so effective. Where degradation is not inhibited at all, as in the case of AB2472 and B_{s-1}, the DNA is originally repaired at about the same rate as in strains with inhibition, but the repaired DNA is degraded, so the fraction repaired begins to fall.

The fact that we find repaired DNA to be degraded about as efficiently as newly irradiated DNA, even though it contains far fewer single-strand breaks, suggests to us that the signal for DNA degradation to begin is not simply a single-strand break.

The question arises as to whether the induced repair process involves a new enzyme which additionally causes repair. Our experiments can be explained without such an additional enzyme. Existing enzymes able to work on DNA inhibited from degradation by the inducible inhibitor would suffice.

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