Interaction between Ultraviolet Light and γ -Radiation Damage in the Induction of Mutants of *Escherichia coli*: the Effect of Some Modifying Treatments

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SUMMARY

The effect of various modifying agents has been studied on the synergistic interaction of u.v.- and γ -radiation in the induction of prototrophic revertants of tryptophan-requiring *Escherichia coli* B/r WP2. The component of γ -radiation damage involved in the interaction shows a large effect of oxygen during irradiation (dose modifying factor of up to 3) and is thus more like lethal than mutational damage. The interaction mutants respond to various post-irradiation treatments in the same way as u.v.-induced, but not γ -induced, mutants, i.e. they are photoreactivable, their frequency is increased by acriflavine and nutrient broth after irradiation and they are susceptible to mutation frequency decline in minimal medium. It is postulated that damage induced in DNA by γ -radiation inhibits the repair of premutational lesions induced by u.v. thus increasing the yield of u.v.-induced mutants. The possible nature of the system involved in this repair (which is not that responsible for the excision of thymine dimers from DNA) is discussed.

INTRODUCTION

In the preceding paper (Davies, Arlett, Munson & Bridges, 1967) we have shown that more prototrophic revertants of the tryptophan requiring strain *Escherichia coli* WP2 are induced by combined treatments with ultraviolet light (u.v.) and γ -radiation than would be expected if the effect of the treatments were merely additive. The additional mutants, which we have termed interaction mutants, have been shown to arise by the interaction of u.v.- and γ -radiation damage during post-irradiation development. For the purpose of this paper the frequency of interaction mutants per survivor is defined as the number of mutants per survivor induced by a combined u.v.- γ treatment less the number of mutants per survivor induced by each treatment given separately to similar bacterial suspensions. In this paper we record the effect on the yield of interaction mutants of various modifying treatments which are known to affect either the initial amount of γ -radiation damage (e.g. oxygen) or the cellular repair processes affecting u.v. damage (e.g. photoreactivating light, acriflavine, nutrient broth). The results indicate that the interaction mutants behave as though they are u.v.-induced.

METHODS

Escherichia coli B/r WP2 was used for most experiments; where stated confirmatory experiments were carried out with *E. coli* WP2 hcr⁻ (Hill) which is deficient in certain dark repair processes.

Microbiological techniques and details of the irradiation sources have been given elsewhere (Davies *et al.* 1967). Glucose-salts medium enriched with 0.75 µg./ml. tryptophan and solidified with 1.5% agar was used for all experiments except those involving mutation frequency decline when enrichment was made with 2.5% Oxoid nutrient broth instead of tryptophan.

Fig. 1

Fig. 2

Fig. 1. Effect of presence (▽) or absence (△) of oxygen during γ -irradiation on the production of interaction mutants after 67.5 ergs mm.⁻² u.v.

Fig. 2. Effect of photoreactivation after 45 ergs mm.⁻² u.v. on subsequent yield of interaction mutants with γ -radiation. ▽, no photoreactivation; △, with photoreactivation.

The source of photoreactivating light was a 500 W photoflood lamp. Suspensions of bacteria in the glucose-salts medium were placed for 20 min. at 10 cm. from the lamp in a water-bath maintained at 15°. Non-photoreactivated controls were wrapped in aluminium foil during an identical treatment.

Acriflavine was obtained from General Biochemicals.

RESULTS

Effect of oxygen

When a dose of u.v. was followed by a range of γ doses given with either oxygen or 'oxygen-free' nitrogen bubbling through the suspension, interaction mutants in *Escherichia coli* B/r WP2 were induced as shown in Fig. 1. The slope of the response curve under oxygenated conditions varied from 1.5 to 3 times that under anoxic conditions in four different experiments.

Fig. 3. (a) Effect of 3 μ g./ml. acriflavine in the plating medium and u.v. on the lethal action of γ -radiation on *Escherichia coli*. $\circ$, no acriflavine; $\bullet$, acriflavine; $\bullet$, 67.5 ergs mm.⁻² u.v. on acriflavine plates; Δ , 67.5 ergs mm.⁻² u.v. followed by γ -radiation, on acriflavine plates. (b) Yield of interaction mutants by 67.5 ergs mm.⁻² u.v. followed by a range of doses of γ -radiation. ∇ , no acriflavine; Δ , 3 μ g./ml. acriflavine in plating medium.

Fig. 4. (a) Lethal effect of u.v. on *Escherichia coli* plated on medium with ($\bullet$) and without ($\circ$) 3 μ g./ml. acriflavine. (b) Yield of u.v.-induced mutants plated on medium with ($\bullet$) and without ($\circ$) 3 μ g./ml. acriflavine.

Effect of photoreactivating light

When a photoreactivating treatment was given between a u.v. dose and a γ -radiation dose, the rate of induction of interaction mutants in *Escherichia coli* B/r WP2 was reduced by a factor of about 8 (Fig. 2). This also happened with *E. coli* WP2 hcr⁻ where, in addition, it was shown that the photoreactivating treatment was effective even when given after the γ -irradiation.

Effect of acriflavine

The addition of acriflavine to the plating medium is known to enhance the lethal and mutagenic action of u.v. (Witkin, 1963) but not the mutagenic action of γ -radiation (Bridges & Munson, 1964). Our experiments, using 3 $\mu\text{g./ml.}$ acriflavine,

Fig. 5. Decline of mutation frequency with time as revealed on broth-enriched plates when bacteria are held in glucose salts medium at 37° after irradiation with 195 ergs mm.⁻² and/or 4 krad. γ -radiation. ○, total induced mutants after u.v. and γ -irradiation; ▲, total induced mutants after u.v. and γ -irradiation less those induced by γ -radiation alone; ●, mutants induced by u.v. alone; ▽, interaction mutants, i.e. total mutants induced by combined u.v. and γ -irradiation less those induced by u.v. and γ -radiation alone. In (a) absolute mutation frequencies are plotted, in (b) these have been plotted as a percentage of their values immediately after irradiation.

confirm this and also shown an enhancement of the lethal action of γ -radiation (Fig. 3a) similar to that observed by Alper (1963) with *E. coli* B/H. The induction of interaction mutants was enhanced by acriflavine (Fig. 3b) to roughly the same extent as the induction of u.v. mutants (Fig. 4).

Mutation frequency decline

It is a well-established fact, dating from the observations of Witkin (1956), that more u.v.-induced mutations appear on glucose+salts plates supplemented with a little nutrient broth than on plates supplemented with tryptophan alone. The amino acids in the broth in some way stabilize the premutational lesions so that more of the latter

are 'fixed'. If u.v.-exposed bacteria are incubated at 37° in minimal medium before plating on plates enriched with broth, the number of mutants appearing rapidly falls until by about 40 min. it is the same as that on minimal plates supplemented with tryptophan alone. This process has been termed 'mutation frequency decline' (Doudney & Haas, 1958); it does not occur with mutations induced by ionizing radiation which are unaffected by the presence of broth (Kada, Brun & Marcovich, 1960; Munson & Bridges, 1966). In Fig. 5*a* we show mutation frequency decline of all the mutants induced by a combined u.v. and subsequent γ -radiation treatment, of u.v. mutants alone, and of interaction mutants. It is clear that interaction mutants are stabilized on plates enriched with nutrient broth immediately after irradiation, and in the absence of broth show the phenomenon of mutation frequency decline. In Fig. 5*b* curves for u.v. mutants, interaction mutants, and u.v. and interaction mutants together, have been re-plotted as a percentage of their value at zero time. Neither the rates of mutation frequency decline nor the ratios of final to initial frequencies are significantly different for the three curves.

DISCUSSION

The oxygen effect experiments may throw some light on the component of γ -radiation damage which is involved in this interaction between u.v. and ionizing radiation damage. The oxygen enhancement ratio for lethality under the conditions of these experiments is about 3 and for mutation induction about 1.3 (Bridges & Munson, 1964). For the production of interaction mutants it varied between 1.5 and 3 which might indicate that straightforward γ -radiation-induced mutations are not involved in the interaction but possibly the same sort of damage which is involved in lethality.

The fact that the u.v. component involved in interaction is photoreactivable does not necessarily imply that thymine dimers are involved since Witkin (1964) has shown that u.v.-mutagenesis may be photoreactivable even in strains known to lack the photoreactivating (dimer-splitting) enzyme. In such a case photoreactivation appears to involve the same indirect mechanism (similar to that involved in photo-protection) which can remove lethal damage (probably including thymine dimers) under certain conditions (Kondo & Jagger, 1966; Kondo & Kato, 1966). The sensitivity of the u.v.-interaction component to photoreactivation after both the u.v. and γ doses have been given is, of course, a further demonstration that interaction occurs during the post-irradiation period, as is also the post-irradiation sensitivity to acriflavine. The enhancing effect of acriflavine on u.v. mutagenesis is believed to be due to an inhibition of excision-repair and/or mutation frequency decline and the enhanced yield of interaction mutants on acriflavine plates confirms the conclusion of Davies *et al.* (1967) that the u.v.-interaction component is also sensitive to these processes.

A direct demonstration that interaction mutants are subject to mutation frequency decline (and thus behave like mutants induced by u.v. alone but not γ -radiation alone) comes from the experiment shown in Fig. 5. It is clear that the proportion of interaction mutants to u.v.-induced mutants is the same in samples plated immediately after irradiation on plates enriched with nutrient broth (which provides partial stabilization against mutation frequency decline) as on those plated after 40 min. in minimal medium (when mutation frequency decline is complete). In addition the rate of decline is the same for interaction as for u.v.-induced mutants.

The slope of a mutation frequency decline curve depends both on the rates of removal and of fixation of premutational damage. The experiment shown in Fig. 5 makes it unlikely that interaction mutants result from the interference by γ -radiation with the rates of either of these processes.

So far then it appears that the γ -component of the damage involved in interaction may be more like lethal than mutational damage, and that the u.v.-component behaves like u.v. mutational damage. The most reasonable interpretation of these results is that damage induced in DNA by γ -radiation inhibits the repair of premutational lesions induced by u.v.

It is not possible at present to state the precise nature of the γ -radiation component of damage. There is evidence that both *Escherichia coli* B/r wp2 and *E. coli* wp2 hcr⁻ can repair a large proportion of lethal ionizing radiation damage, including possibly single strand breaks (Bridges & Munson, 1966, McGrath & Williams, 1966), but it is not known whether the lesions which are ultimately lethal are unrepaired single strand breaks or of a further unidentified type.

There is general agreement that pyrimidine dimers are involved in the induction of mutations by u.v. alone, whether as the sole type of lesion (Hill, 1965; Kondo & Kato, 1966) or in conjunction with further unidentified lesions (Witkin, 1964, 1966; Doudney, 1963, 1966; Bridges, 1966). One of the strongest arguments against pyrimidine dimers being the only lesions involved is the fact that the removal of pre-mutational lesions by the mutation frequency decline system is severely inhibited by the presence of nutrient broth, whereas the removal of thymine dimers is stimulated (Setlow & Carrier, 1964).

If we are to explain our mutation interaction results in a way similar to that used by Haynes (1964) to explain his lethal interaction results, we must do so in terms of a repair system able to remove premutational lesions induced by u.v. and inhibited by γ -radiation lesions (which might imply an ability to repair certain γ -radiation lesions). So far two candidates appear as possible contenders for the role of this hypothetical repair system.

The first has been described in terms of a low temperature mutation loss (l.t.m.l.). When minimal plates enriched with tryptophan are incubated at temperatures below about 16° after irradiation, fewer mutants appear compared with plates incubated at 37°, whether after γ -radiation (Bridges & Munson, 1964) or u.v. (Munson & Bridges, 1966).

While this l.t.m.l. might seem at first sight unlikely to be associated with u.v.- γ interaction at 37°, closer examination shows that this need not be so. The phenomenon of l.t.m.l. has been ascribed to a differential temperature effect on the rates of repair and fixation of premutational lesions (Bridges & Munson, 1964), but since at temperatures above about 22° the rates of both repair and fixation apparently share the same dependence upon temperature it follows that the repair may still go on to an appreciable extent at 37° although we have no means of detecting it. L.T.M.L. is also a suitable candidate for the interaction effect as it occurs in both hcr⁺ and hcr⁻ strains to a similar extent and does not appear to be related to excision repair or mutation frequency decline.

The second presumed repair system for u.v. damage (K-reactivation) has been described by Kneser (1965, 1966) as being present in wild-type strains of *Escherichia coli* K12 and B/r, absent in 'recombination-less' K12 and inhibited in *E. coli* B and

Bs-1. Since it is present in both the hcr⁺ and hcr⁻ strains which we have used we cannot tell whether it is involved in our interaction phenomenon and, although there is evidence that it operates on both u.v. and γ -radiation lethal damage, it may act on non-genetic components of radiation damage. In some early work, Demerec & Latarjet (1946) showed that the rate of mutation induction to phage resistance was the same in *E. coli* B and B/r which indicates that the premutational damage involved there was not amenable to K-reactivation. The same conclusion has been reached by Dr E. M. Witkin (personal communication) for reversion to prototrophy. It is possible that K-reactivation is the system involved in the u.v.- γ lethal interaction observed by Haynes (1966) since a similar interaction occurs with *E. coli* WP2 hcr⁻ which possesses K-reactivation (Bridges & Munson, unpublished observation) but not *E. coli* Bs-1 (Haynes, 1964) which does not. It would obviously be desirable to examine whether synergistic interaction for mutation induction is also absent in *E. coli* Bs-1, as this would give an indication of the possible role of K-reactivation in the mutant interaction phenomenon.

If, however, neither l.t.m.l. nor K-reactivation is involved in interaction of mutational damage, it would presumably be necessary to postulate a further repair system.

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Note added in proof

An interaction between u.v. and X-irradiation has recently been reported by Kada, Doudney & Haas (1966) (*Mutation Res.* **3**, 118) in *E. coli* B/r WP 2. Interaction occurred only when there was a period of incubation at 37° between the two irradiations and appeared to depend on RNA and protein synthesis. Furthermore, minimal plates without enrichment were used so that most mutants (both u.v. and X-ray induced) would not be expressed. We consider that the interaction observed under these conditions is probably concerned with the expression of mutations in the absence of tryptophan and is a quite different phenomenon from that which we have examined.