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Effect of UV Irradiation on Transduction by Coliphage T1

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Lysates of the virulent coliphage T1 transduce seven markers between strains of *Escherichia coli* with reproducible efficiencies which range from 10^{-7} to 10^{-5}. The ability of a UV-irradiated lysate to transduce Arg^+, Str^R, Trp^+, Lac^+, and Pro^+ is 90% and Bio^+ is 99% inactivated by doses which inactivate plaque formation of T1 by six orders of magnitude. A dose of irradiation which causes a 1- to 2-log drop in the titer of T1 stimulated Gal^+ transduction by two- to three-fold; no other marker tested was stimulated. Irradiation causes dislinkage of some co-transducible markers but not others.

The effect of UV irradiation on the ability of a phage lysate to mediate generalized transduction has been studied by many investigators beginning with Zinder (7) and Garen and Zinder (4). Perhaps the most quantitative study of the effects of UV irradiation on a phage lysate's ability to transduce has been that of Benzinger and Hartman (1).

It has been determined that the ability of a transducing phage to mediate the transduction of bacterial genes is relatively more resistant to inactivation by irradiation than the plaque-forming ability of the same phage. In many reports, it has been shown that a dose of UV irradiation which can cause a reduction of several orders of magnitude in plaque-forming ability often stimulates the transduction of bacterial markers (1, 4-7).

In this report the ability of the virulent coliphage T1 to transduce a variety of markers both before and after UV irradiation is studied. The results show that, with but one exception, irradiation of T1 lysates does not cause a stimulation of transduction.

MATERIALS AND METHODS

Strains and media. All but a few of the bacterial and phage strains and media have been described in previous reports (2, 3). Therefore, only the briefest descriptions are given here. The amber (*am*)-permissive (Su^+), streptomycin-resistant (Str^R), prototrophic donor used was *Escherichia coli* KB-3. Two recipients were used. The amber-nonpermissive (Su^-) *E. coli* strain W3350 is unable to ferment galactose (Gal^-) or lactose (Lac^-) and is sensitive to streptomycin (Str^S). Variants of W3350 which, respectively, were unable to synthesize biotin (Bio^-), arginine (Arg^-), tryptophan (Trp^-), or proline (Pro^-) were also used. The *E. coli* strain W3110, which is Su^- and contains the mutations *trp*A-9761 and *cys*B, was kindly provided by C. Yanofsky; this strain can not synthesize cysteine (Cys^-) or tryptophan. Phage P1-1127, a nonlysogenizing mutant of the temperate phage P1, was kindly provided by P. Harriman. T1*am* is $T1am_8am_{11}$.

As mentioned, the media used were identical to those described previously (2, 3) with the following exceptions: synthetic agar (TMA) containing galactose as the sole source of carbon and energy was used to select for co-transduction of Gal^+ and Bio^+. TMA supplemented with cysteine was used to select for the transduction of W3310 for Trp^+; TMA supplemented with tryptophan was used to select for the transduction of W3310 for Cys^+; TMA with no added cysteine or tryptophan was used to select for the co-transduction of W3310 for Cys^+ and Trp^+.

Transduction. Experiments were performed as previously described (2, 3) with one exception. To obtain reproducible results for the transduction of W3350 for Bio^+, fewer than 2×10^7 cells must be spread on the minimal selective plates. The co-transduction of Gal^+ and Bio^+ is not significantly different whether one selects only for Gal^+ (on eosin methylene blue plates containing biotin and galactose), only for Bio^+ (on TMA plates with glucose as the sole source of carbon and energy), or for both Gal^+ and Bio^+ (on unsupplemented TMA plates containing galactose in place of glucose). Similarly, there is no selection dependence for the co-transduction of Cys^+ and Trp^+.

Irradiation of lysates. T1*am* lysates were diluted into 0.1 M (pH 7.2) Tris buffer to an appropriate concentration (less than 10^9 per ml) and dispensed in 3- to 4-ml samples to glass petri dishes. The individual samples were irradiated with UV light at a distance of 60 cm by a 15-watt General Electric Germicidal lamp which had been switched on at least 15 min before irradiation. A General Electric wattmeter indicated that the lamp emitted 12.9 ergs/mm^2 per s at the distance used. All manipulations which resulted in the infection of cells with irradiated phage (such as titering or transductions) were performed only in the

presence of yellow light. The irradiated samples were stored in the refrigerator in tightly stoppered tubes.

RESULTS

Generalized transduction by T1. T1 can transduce a variety of markers (2). It is demonstrated here that, with rare exceptions, any given marker is transduced reproducibly from KB-3 to W3350 within a two- to three-fold range even when different lysates are utilized (Table 1). Of the 42 experiments used to compile Table 1, only one experiment (a tryptophan transduction) fell outside the two- to three-fold range of reproducibility.

UV irradiation of lysates. Lysates of T1*am*·Su$^+$, i.e., T1*am* grown on strain Su$^+$, were subjected to UV irradiation for various periods of time under the conditions described above. Since phage T1*am* kills Su$^-$ cells, only singly infected cells can become transductants. However, irradiation can block the killing effect of T1*am*. Therefore, at high multiplicities some effects of UV irradiation may occur because cells which obtain both a transducing particle and a T1*am* particle are able to survive and become transductants. To reduce the number of multiply infected cells to an insignificant fraction of the total number of infected cells, we did not use multiplicities of infection greater than 0.2. The multiplicity was calculated by using the phage titer before irradiation.

Exposure of T1 to UV light causes the plaque-forming ability to be reduced exponentially at a rate of approximately one order of magnitude per minute of exposure (results not shown). As was noted in a previous report (2), the effect of irradiation on T1's ability to transduce is less sensitive to UV inactivation.

The effects of irradiation on the ability of T1*am*·Su$^+$ to transduce Str^R, Arg$^+$, and Trp$^+$ are nearly identical. Figure 1 shows the effects of irradiation on the efficiency of transduction (EOT) of Str^R. The EOT of all three markers is nearly insensitive to exposures of irradiation up to 2 min. Even after 6 min of irradiation, the EOT of any of these three markers declines only about one order of magnitude. The EOT of Lac$^+$ and Pro$^+$ by unirradiated T1*am*·Su$^+$ is about one-third that of Str^R, etc.; however, the inactivation rate is about the same (Fig. 1 shows the results for Pro$^+$). The data suggest that the inactivation rate of Bio$^+$ is greater than that of Str^R, Pro$^+$, etc., but the effect is not striking. Differing from the other six markers, transduction of Gal$^+$ shows a two- to three-fold stimulation after 1 to 2 min of irradiation but thereafter declines at the same rate as most of the other markers.

Co-transduction. It has been reported (1, 5) that UV irradiation of transducing lysates reduces the probability that a cell transduced for a given marker will be co-transduced for a closely linked marker. Presumably, UV-stimulated recombination increases the probability of a crossover event between linked markers. To determine the effect of UV irradiation of T1 lysates on the probability of linked transduction, the co-transduction of Cys$^+$ and Trp$^+$ on the one hand, and of Gal$^+$ and Bio$^+$ on the other hand, was studied.

It was found (Table 2) that the fraction of Cys$^+$ transductants which were also transduced for Trp$^+$ showed no significant change when lysates were irradiated either 2 or 4 min; this is shown by the fact that the ratio of the efficiency of co-transduction (EOCt) to the EOT of Cys$^+$ remained nearly constant at 0, 2, and 4 min of irradiation. Likewise, the ratio of EOCt to EOT-Trp$^+$ does not fall after irradiation; the slight rise in the EOCt/EOT-Trp$^+$ ratio is probably not significant. Therefore, UV irradiation of a T1 lysate does not reduce the linkage of Cys$^+$ and Trp$^+$.

The effect of UV irradiation on the linkage of

TABLE 1. *The EOT of W3350 for various markers by T1am grown on KB-3*[a]

Marker transduced	No. of lysates	No. of experiments	Range of EOT		Average EOT
			Lowest value	Highest value	
Bio$^+$	5	9	1.0×10^{-5}	3.4×10^{-5}	1.6×10^{-5}
Str$^+$	3	5	9.6×10^{-7}	3.2×10^{-6}	2.0×10^{-6}
Arg$^+$	4	5	1.0×10^{-6}	2.2×10^{-6}	1.2×10^{-6}
Trp$^+$	5	5	6.0×10^{-7}	2.7×10^{-6}	1.2×10^{-6}
Pro$^+$	4	6	2.6×10^{-7}	4.9×10^{-7}	4.0×10^{-7}
Lac$^+$	4	4	2.5×10^{-7}	6.5×10^{-7}	3.9×10^{-7}
Gal$^+$	7	8	1.7×10^{-7}	5.4×10^{-7}	3.4×10^{-7}

[a] Auxotrophic variants of *E. coli* strain W3350 Su$^-$ were infected with T1*am*·KB-3Su$^+$ at a multiplicity of infection of 1 or less. After a short incubation at 35 C, the infected cells were placed on an appropriate selective or differential medium (see text). After several days incubation, the plated were scored for transductants.

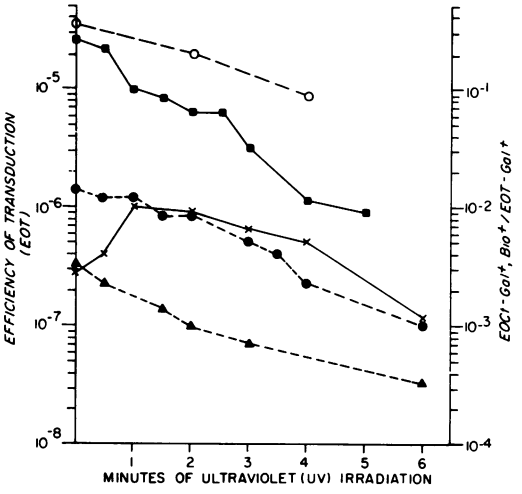

FIG. 1. *Samples of T1am·Su⁺ lysates were irradiated for various periods of time and then used to infect auxotrophic variants of the Su⁻ receipient strain at multiplicities of less than 0.2. After a short incubation at 35 C, the infected cells were placed on an appropriate selective or differential medium and incubated at 35 C for several days. Each of the markers listed in Table I was tested from at least two different irradiated lysates and the average EOT for a given time of irradiation is plotted. The data for Arg⁺, Str^R, and Trp⁺ were so nearly identical that only the data for Str^R is given in the figure. Likewise, the data for Lac⁺ and Pro⁺ were so similar that only the data from the Pro⁺ experiments are plotted. Bio⁺, ■; Str^R, ●; Pro⁺, ▲; Gal⁺, X; EOCt-Gal⁺, Bio⁺/EOT-Gal⁺ (i.e., the ratio of the EOCt of Gal⁺ and Bio⁺ to the EOT of Gal⁺, O).*

Gal⁺ and Bio⁺ is more complex than the effect on Cys⁺ and Trp⁺. The ratio EOCt/EOT-Gal⁺ shows a decrease in linkage with increasing irradiation (Table 2). If the logarithim of the ratio is plotted against the time of irradiation (Fig. 1), the decrease in linkage is observed to be approximately linear. This observation is nearly identical to the exponential decrease in linkage reported by others for phage P22 (1). The EOCt/EOT-Bio⁺ ratio rises 20-fold after 2 min irradiation and decreases upon continued irradiation (Table 2).

Thus the fraction of Gal⁺ transductants which are also transduced for Bio⁺ decreases while the fraction of cells transduced for Bio⁺ which in addition obtain Gal⁺ increases.

Transduction of W3350 and W3110 by P1·KB-3. The results for the transduction of markers by P1 were so similar to those reported by other investigators (5, 6) that the data are not given. The results can be summarized as follows: irradiation of P1·KB-3 lysates for 0.5 to 2.0 min causes a 2- to 20-fold increase in the markers tested. The exact increase depended on the marker tested. Therefore, using the same donor and the same recipients as were used with T1, it was observed that the effect of UV irradiation on the ability of a phage lysate to transduce differs with the phage employed.

Irradiation of P1·KB-3 lysates led to a nearly exponential decrease in linkage of Cys⁺ and Trp⁺, a result similar to the observations of others (1).

DISCUSSION

When T1*am* is grown on amber-permissive, prototrophic donors the resulting lysates are able to transduce a variety of markers to nonpermissive, auxotrophic recipients. The EOT of the markers studied varies between 10^{-7} and 10^{-5}. For any one of the seven markers studied, the EOT is reproducible within a two- to three-fold range even when different lysates are utilized (Table 1).

Unlike other transducing systems (1, 5–7), the UV irradiation of T1*am* lysates does not, as a rule, result in a stimulation of transduction. The ability to transduce most markers (Str^R, Arg⁺, Trp⁺, Pro⁺, and Lac⁺) is reduced approximately 90% by a dose of irradiation which causes a drop of six orders of magnitude in the plaque-forming ability of the phage (Fig. 1). However, the effect of irradiation is complex and the ability to transduce the Bio⁺ marker seems more sensitive to inactivation than that of the five markers just mentioned while the

TABLE 2. *Effect of UV irradiation on the linkage of co-transducible markers*[a]

Min of UV irradiation	EOCt-Cys⁺, Trp⁺/EOT-Cys⁺	EOCt-Cys⁺, Trp⁺/EOT-Trp⁺	EOCt-Gal⁺, Bio⁺/EOT-Gal⁺	EOCt-Gal⁺, Bio⁺/EOT-Bio⁺
0	0.10	0.14	0.36	0.002
2	0.11	0.19	0.25	0.04
4	0.06	0.24	0.09	0.009

[a] Samples of T1*am* lysates which had been grown on KB-3 Su⁺Cys⁺Trp⁺Gal⁺Bio⁺ were irradiated for 0, 2, and 4 min, respectively, according to the procedure given in text. The samples were used to infect either W3110 Su⁻Cys⁻Trp⁻ or W3350 Su⁻Gal⁻Bio⁻ at multiplicities of less than 0.2 (multiciplicity of infection based on unirradiated samples). After a short incubation period, infected cells were plated on selective medium as described in text. The ratio of the EOCt of two markers (e.g., Cys⁺ and Trp⁺) to the EOT of one of the markers (e.g., Cys⁺) is a measure of the effect of irradiation of linkage (see reference 1). The values for the EOCt-Gal⁺, Bio⁺/EOT-Gal⁺ ratios are also presented graphically in Fig. 1.

transduction of Gal$^+$ is stimulated two- to three-fold by mild irradiation (Fig. 1).

The lack of stimulation for most markers is puzzling and it is difficult to understand why bacterial DNA packaged by T1*am* should be stimulated to transduce while DNA carried by other phages, for example P22 or P1, is stimulated. With P22 it is believed (1) that complete or stable transductants arise from a pool of abortive or unstable transductants. By causing an increase in recombination between the unstable, unintegrated DNA which mediates abortive transduction and the bacterial chromosome, it is thought that irradiation causes a shift from abortive to complete transduction. If this model is correct then except for the Gal$^+$ marker it might be expected that T1 does not cause abortive transduction; up to the present we have not been able to detect abortive transduction by T1*am* for any marker.

The only simple models we are able to propose to explain why most T1*am*-mediated transduction is not stimulated by irradiation are: (i) during infection by T1 the UV-sensitive sites in the bacterial DNA are altered in such a way as to make them resistant to UV-stimulated recombination, or (ii) T1 modifies the bacterial DNA it packages in such a way as to make the probability of transduction by T1 transducing particles nearly 100%. In either case, irradiation would have little or no stimulatory effect on transduction. The results obtained for the co-transduction of Cys$^+$ and Trp$^+$ (Table 2) support the first hypothesis since any stimulation of recombination by UV irradiation between Cys$^+$ and Trp$^+$ should lead to dislinkage of the markers. The fact that P1-mediated co-transduction of Cys$^+$ and Trp$^+$ is reduced by irradiation indicates that UV stimulation of recombination between the two markers is possible, and further indicates that when the region between Cys and Trp is packaged by T1 it is altered.

The transduction of either Gal$^+$ or Bio$^+$ is affected differently by UV irradiation than the other markers tested. The transduction of Gal$^+$ is stimulated two- to three-fold by mild (2 min) irradiation whereas Bio$^+$ transduction seems to be more sensitive to inactivation by irradiation than the other markers. Furthermore, although the two markers are linked, Bio$^+$ is transduced with the highest efficiency of the markers tested whereas Gal$^+$ is among the markers which are least efficiently transduced. Finally, the fraction of Gal$^+$ transductants which are co-transduced for Bio$^+$ decreases after irradiation whereas the fraction of Bio$^+$ transductants which receive the Gal$^+$ marker increases.

From the data in this report we can not give a definitive explanation of the unusual (compared to other markers) behavior or Gal$^+$ and Bio$^+$ transduction. However, several observations seem to suggest a partial solution to the problem. It seems evident that at least one site (perhaps more) which is stimulated in recombination by UV irradiation remains between Gal$^+$ and Bio$^+$. This site(s) can account for both the stimulation of Gal$^+$ transduction and the dislinkage of Gal$^+$ and Bio$^+$. When selection for Gal$^+$ (alone or together with Bio$^+$) is used to score transductants, the co-transduction of Gal$^+$ and Bio$^+$ strongly resembles co-transduction in other systems (for example by using P1 to transduce our strains or transduction by P22 (1). However, selection of Bio$^+$ alone leads to results which simply can not be explained on the basis of data presented in this report.

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