EFFECT OF IRRADIATION ON TRANSDUCTION AND LYSOGENISATION IN *SALMONELLA TYPHIMURIUM*

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ABSTRACT

Irradiation of either temperate Phage 547 of *Salmonella* or recipient cells increases the frequency of transduction whereas lysogenization decreases. The increase in transduction is owing to the stimulating effect of the irradiation on recombination and loss of lytic action of the phage. The irradiation results suggest that the whole phage genome is more radiosensitive than the transducing fragment.

INTRODUCTION

IN the previous paper[1] it has been reported that wild type *S. typhimurium* strain 547 is lysogenic and releases temperate phage called P 547. This phage belongs to the class of general transducing temperate phages of *S. typhimurium* as it can transduce any marker. Transduction and lysogenization are shown to be independent processes in this case, unlike in lambda phage-*E. coli* system.

Zinder[2] studied the effect of irradiated phage P 22 of *Salmonella* on transduction and found a rise in the frequency as compared to that of unirradiated phage. Similarly, an enhancement of sexual recombination was found by irradiation of the F^+ donor parent cells[3]. I wish to report in this paper the results of the effect of irradiated 547 phage and irradiated recipient cells on the efficiency of transduction and lysogenisation. It was hoped that this study might give some information on the nature of transducing fragment and phage genome.

MATERIALS AND METHODS

The bacterial cultures, phage stock, media, and the techniques for transduction and lysogenization are described in the earlier paper[1].

RESULTS

Effect of gamma irradiation on phage survival and adsorption.—A suspension of 547 wild type phage in BHI (Brain heart Infusion broth) was irradiated at 200 kr, 400 kr and 600 kr in plastic tubes attached onto the window of Cobalt-60 radiation source. The ability of plaque formation on sensitive *S. typhimurium* (TC) cells and adsorption (on heat killed *S. typhimurium* 533 cells) was measured in control and irradiated phage samples. The effect of various doses of irradiation on per cent survival and adsorption are shown in Table I. The data showed that although there was a linear decrease in per cent survival of phage, when phage was irradiated at various doses, the adsorption remained to be 100% at these doses indicating that irradiation inactivated the phage genome which did not affect on the adsorption capacity.

TABLE I

Effect of gamma irradiation of 547 phage on survival, adsorption and its efficiency in transduction and lysogenisation

Irradiation dose in kr.	% Survival[a]	% Adsorption[b]	H^+–T^+–L^+ transduction[c]	% Lysogeny[d]
0 (Control)	100·0	100	196·0	90·0
200	38·2	100	259·2	82·0
400	8·8	100	230·5	68·1
600	0·8	100	138·4	46·5

(*a*) Phage suspension with a titre of $6{\cdot}8 \times 10^8$/ml was irradiated and titrated on T.C. cells.
(*b*) The percentage of adsorption of phage was determined on heat killed cells[5].
(*c*) The multiplicity of infection (m.o.i.) was kept 0·3 and the number of transductions are expressed per 10^8 recipient cells.
(*d*) The percentage of lysogenisation was determined among the transduced cells.

Effect of irradiated phage on transduction.—The above irradiated samples were used for transduction experiments to see the correlation between the phage survival or inactivation, adsorption and ability to transduce by the irradiated phage. The frequency of general transduction (for His^+, Try^+, and Leu^+ markers) by control and irradiated 547 phage is shown in Table I. It is quite evident that irradiation of phage with 200 kr and 400 kr enhances the frequency of transductions as compared to unirradiated phage. Garen and Zinder[4] found a similar effect of U.V. irradiated phage P 22 on transduction of these markers. The transduction frequencies have decreased below control level when the phage was irradiated at 600 kr dose (Table I). These results demonstrate that

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inactivated phage which has not lost the capacity of adsorption could transduce efficiently, *i.e.*, an active phage genome is not necessary for adsorption and transduction processes. Since the adsorption of irradiated phage is not increased, it is probable that irradiation itself makes the transduction process more efficient. To test this view, the transduction experiments were performed in which the U.V. irradiated recipient cells and unirradiated phage were employed.

Effect of U.V. irradiation on survival of recipient cells and their ability to adsorb phage.—First the rate of inactivation of recipient cells by U.V. irradiation and their capacity to adsorb phage were determined, the results of which are shown in Table II. After 60 and 90 seconds of irradiation,

TABLE II

Comparative data on the effect of U.V. irradiation on survival of recipient cells, and their ability in adsorption and transduction

U.V. dose in seconds	% Survival	% Adsorption	Transductions per 10^8 cells
0 (Control)	100·0	100·0	517·3
60	26·2	100·0	886·0
90	13·7	100·0	593·0

Exponentially growing culture with a titer of $2{\cdot}4 \times 10^9$/ml was suspended in chilled saline and was irradiated at a distance of 53 cm, with a 15 watt General Electric Germicidal lamp for above indicated times. All operations were carried out under red light to avoid photoreactivation. The above cultures were used for transduction experiments in which the m.o.i. was 2·3 in all the cases.

the cells were titrated for survival and the same cells were used for phage adsorption measurements. A control culture was run in parallel for comparsion. The results in Table II show that 26·2% and 13·7% of cells survived when they were irradiated for 60 and 90 seconds. However, these cultures containing the mixture of active and inactive cells possessed the same capacity of phage adsorption as control. These results are analogous to those obtained by using heat killed cells which could still adsorb the phage without supporting replication[5, 6].

Effect of U.V. irradiated recipient cells on transduction.—The transduction experiments were carried out by using the above irradiated recipient cells and non-irradiated 547 phage. The data in Table II indicate that the transduction frequency has significantly increased (as it did for irradiated phage) when the indicator cells were irradiated for 60 and 90 seconds. The frequency of transduction was lower in the case of 90 seconds irradiation than for 60 seconds, although both these values were much higher than the control. The reason for this decrease was that survival of recipient cells was lower at 90 seconds than at 60 seconds of irradiation (Table II). Thus the above results (in Tables I and II) indicated that irradiation of either phage or recipient cells enhanced the process of transduction without an increase in phage adsorption.

Effect of irradiation on lytic action of phage and it's effect on transduction.—It is well established that in the temperate phage lysate only a small fraction of phage particles will constitute transducing phages and other fraction of the phages will either lysogenise or lyse the infected cells[7]. Watson[8] has shown that X-irradiation of T_2 phage concurrently diminishes lytic action and phage survival. The decrease in lytic action of irradiated temperate phage may be one of the factors in increasing transduction frequency in the preceding experiments (Table I). If the lytic action of phage is decreased by irradiation one can expect a higher percentage of survival of irradiated phage-infected cells than unirradiated phage-infected cells. To test this possibility the following experiment was performed to determine the lytic or killing action of control and irradiated phage on recipient cells by measuring the cell survival before and after infection.

The cells were allowed to infect with 200 kr, 400 kr and 600 kr irradiated and unirradiated phage samples and after 20 minutes of infection the unadsorbed phage was removed by centrifugation. The cell assays were made before and after infection of phage. The results in Table III show that

TABLE III

Comparative data on the per cent survival of irradiated phage and cell survival after infection

Irradiation dose in kr	% Phage survival	% Survival of infected cells	% Lysis of infected cells
0 (Control)	100·0	68·6	31·4
200	38·2	74·0	26·0
400	8·8	84·6	15·4
600	0·8	100·0	0·0

The m.o.i. was 0·36 in all four cases.

the per cent survival of infected cells increased as the function of irradiation dose, that is the decrease in cell lysis after infection with irradiated phage approximately paralleled the decrease in phage

survival after irradiation. From these results it appeared that the gamma-irradiation affected the lytic or killing property and the viability of 547 phage to the same degree. Contrary to this the host killing property of the virulent phage T_2 is enormously greater in its resistance to U.V. irradiation than the viability of the phage[8]. This suggests that lysis of the cells by temperate phage may require phage reproduction but in the case of virulent phage nonviable phages may also kill or lyse the cells. Due to loss of host killing property of irradiated 547 phage there will be a greater chance for the phage to transduce a cell as compared to unirradiated phage, as a result, the frequency of transduction is increased in the former case.

Effect of irradiated phage on lysogenisation.— The fact that the frequency of transduction is not affected by the irradiated inactive phage, suggests that the genetic material of the phage alone and not the transducing chromosome fragment is inactivated by irradiation particularly at lower doses. If this is so, lysogenisation should decrease in the same order as phage inactivation since lysogenisation is established by the incorporation of the phage genome into bacterial chromosome[9]. In view of this, the effect of irradiated phage on lysogenisation frequency was determined (Table I). Unlike transduction, the frequency of lysogenisation steadily decreased as the irradiation dose increased. These results thus help in distinguishing the phage genetic material from that of the transducing fragment. The transduction and lysogenisation results reveal that transducing fragment is much less sensitive to irradiation than phage genome.

Discussion

Why does gamma or U.V. irradiation of either phage or recipient cells enhance the transduction frequency though there is no increase in adsorption? One explanation suggested by Jacob, Wollman and Hayes[3] is that irradiation has a stimulating effect on the genetic recombination. In *Drosophila* high temperature and irradiation have been shown to stimulate crossing over[10]. The data presented here support the notion that loss of lytic action or host-killing property of the irradiated inactive phage (but transducible) appears to be another important factor in increasing the transduction frequency by way of decreasing the cell lysis of the infected cells.

The data presented in the previous paper[1] that H^+–T^+–L^+ markers were not carried on a single large transducing fragment, but instead each marker was present on a separate fragment. Some further evidence on the nature of the transducing fragment and phage genome may be deduced from the differences on the effect of irradiation on phage inactivation and its ability to transduce and lysogenize. Both phage survival and the lysogenisation decreased as the irradiation dose increased without a corresponding decrease in transduction. According to target theory[11] the smaller the action volume, the more resistant is the particle to irradiation, therefore, it may be suggested that the transducing DNA fragment is smaller than the genome of 547 phage.

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