THE PHOTODYNAMIC ACTION OF PROFLAVINE ON PHAGE T4

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In the presence of a variety of dyes, primarily of the acridine and thiazine groups, phage particles become sensitive to inactivation by visible light (Welsh & Adams, 1954; Yamomoto, 1958; Kaufman & Hiatt, 1959). The mechanism responsible for this photodynamic inactivation (PI) is not clearly understood. With phage T2 and methylene blue the host-killing capacity of the phage is lost at one third the rate of the plaque-forming ability (Welsh & Adams, 1954). Thus some of the inactivations result from the destruction of the mechanism responsible for phage adsorption and possibly injection. The affinity for DNA characteristic of the photosensitising dyes led Yamomoto (1958) to suggest that the phage DNA is the major target for inactivation.

Comparative studies of the inactivation properties of the T-phages provide a clue to the mechanism of PI. The T-odd phages become maximally sensitive to inactivation immediately upon addition of the dye and lose this sensitivity as soon as the dye is removed. The T-even phages, however, become progressively more sensitive with increasing time of exposure to the dye and maintain this sensitivity upon removal of the dye (Yamomoto, 1958; Helprin & Hiatt, 1959). Helprin and Hiatt (1959) proposed that this difference results from the lower permeability of the T-even phage particles to the dye, since

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they are normally sensitive to osmotic shock. This is evidence for the existence, around the phage DNA, of a barrier preventing the rapid diffusion of molecules. The T-odd phages are resistant to osmotic shock and must, therefore, have a more permeable head structure.

If permeability does affect the photodynamic sensitivity of phage by controlling the passage of the dye molecules it would seem that there are sites of inactivation within the phage particle. Thus apart from the effects on adsorption and injection there is possibly an internal site for inactivation involving the binding of the dye to the phage DNA.

This paper is concerned with those inactivation damages which do not affect the adsorbing capacity of the phage. The results show that the photodynamic sensitivity of phages is influenced by the permeability of the phage membrane and that the DNA is a major target for inactivation.

The role of permeability in controlling the rate of PI with proflavine has been examined directly by comparing the sensitivities of the wild type of phage T4 (Benzer, 1955) and an osmotic shock-resistant mutant ($T4o_1$) differing by a single mutation (Brenner & Barnett, 1959). This mutation alters the head protein to produce a more permeable head membrane. For the presence of DNA damage we have examined the ability of photodynamically inactivated phage particles to undergo multiplicity reactivation (MR). MR is the phenomenon whereby two or more phage genomes, each inactive in solo, may cooperate to produce phage progeny when in the same bacterial cell (Luria 1947). Since MR is an intracellular phenomenon and is associated with genetic recombination it is a property of damaged DNA.

Results and Discussion: The photodynamic inactivation of T4 phage particles involves two steps; (a) sensitisation by pretreatment with the dye and, (b) irradiation of the phage-dye complexes with visible light.

Inactivation curves for $T4^+$ and $T4o_1$ pretreated with proflavine before irradiation are shown in Figure 1. Under identical conditions the $T4o_1$

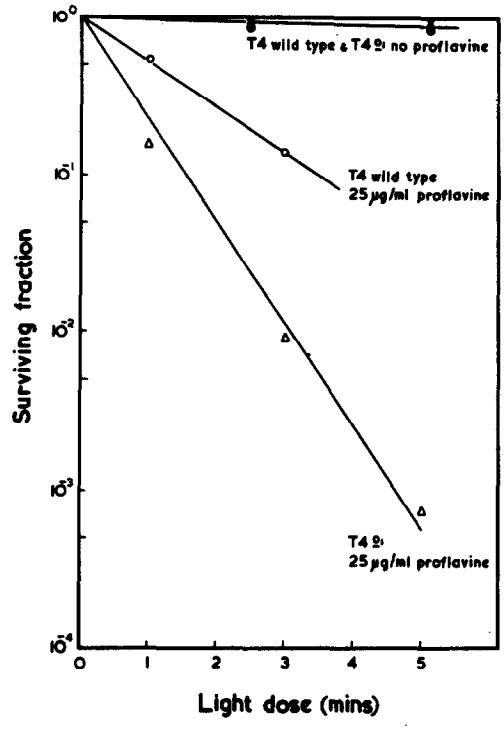

Fig. 1. Buffer suspensions of $T4^+$ and $T4o_1$ (10^8 phage/ml.) were pretreated by incubation in darkness for 30 min. at 37°C. with 25 µg/ml. proflavine hemisulphate (British Drug Houses). Aliquots of 4 ml. were irradiated at a distance of 51 cm. from the visible light source. Surviving phage were assayed on Escherichia coli strain B. Control samples were treated identically except that proflavine was omitted.

The light source and conditions of irradiation have been described previously (Ritchie, 1964). The pH 7.0 phosphate buffer used was the adsorption medium described by Hershey and Chase (1952).

mutant is considerably more sensitive to inactivation than $T4^+$. Since the only variable is the permeability of the phage head protein, we conclude this to be the cause of the different photosensitivities. When pretreated in the absence of proflavine both phages are resistant to visible light.

To examine more closely the effect of permeability on the uptake of the dye, suspensions of $T4^+$ and $T4o_1$ were pretreated with proflavine for various times and then irradiated with a fixed dose of visible light. By this technique it is possible to follow the uptake of the dye. The result is shown in Fig. 2. In both cases the photosensitivity increases with time of

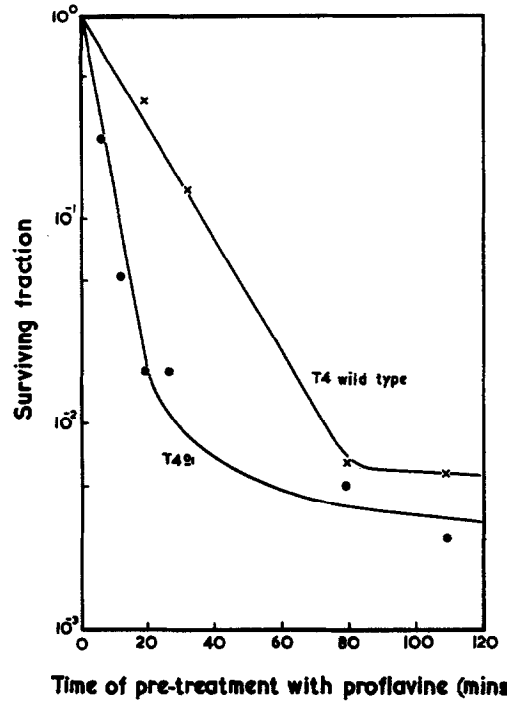

Fig. 2. Suspensions of $T4^+$ and $T4o_1$ at 10^9 phage/ml. in buffer containing proflavine (25 µg/ml.) were incubated in darkness at 37°C. At subsequent intervals samples were diluted 100-fold in buffer and irradiated for 3 min. with visible light. Surviving phage were assayed on E. coli B.

exposure to the dye, however, the increase is much more rapid for the $T4o_1$ phage and maximum sensitivity is reached earlier. The final sensitivity is roughly the same for both phage types.

To study the reversibility of the phage-dye complex, samples of $T4^+$ and $T4o_1$ pretreated with proflavine under the same conditions, were diluted out of proflavine and irradiated with a fixed dose of light at intervals following the dilution. The change in sensitivity following the dilution is plotted in Fig. 3. As the time after dilution increases the sensitivity to inactivation decreases. This decrease is faster in the case of the $T4o_1$ mutant and complete resistance is eventually attained. From the shape of the curve it appears that the $T4^+$ phage will also attain complete resistance.

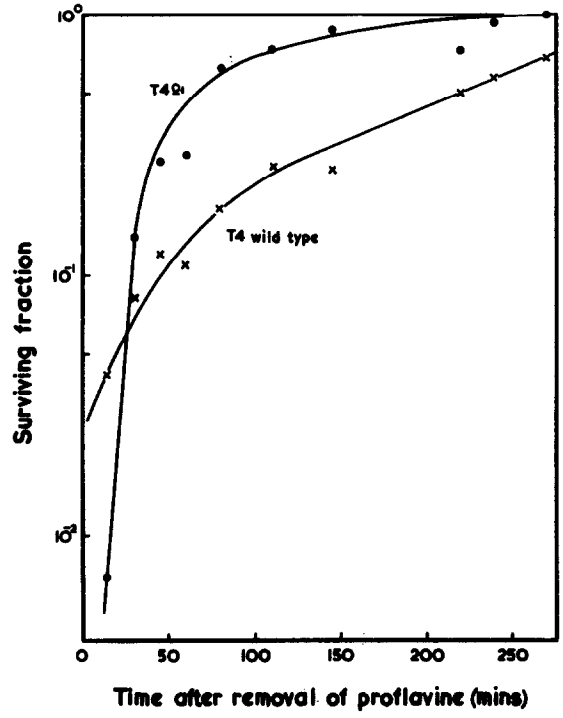

Fig. 3. Suspensions of $T4^+$ and $T4o_1$ at 10^9 phage/ml. in buffer containing proflavine (25 μg/ml.) were incubated in darkness for 60 min. at 37°C. Samples were diluted 1000-fold into buffer and incubated in darkness at 42°C. At intervals following the dilution aliquots were removed and irradiated for 3 min. with visible light and the surviving phage were assayed on E. coli B.

The data presented in Figs. 2 and 3 provide further evidence that the permeability of the phage membrane is an important factor in determining the photosensitivity of a phage; in both experiments the change in photosensitivity is more rapid in the case of the more permeable $T4o_1$ mutant.

Table 1. shows that $T4^+$ particles inactivated by the photodynamic action of proflavine are capable of MR; the phages survive as plaque formers to a much greater extent under conditions of multiple infection than in single infection. This evidence confirms the idea that PI can result from damage to the phage DNA. In support of this result recent evidence has shown that the photodynamic action of acridines can induce mutations in phage T4 (Ritchie,

1964) and in E. coli (Webb & Kubitschek, 1963; Nakai & Saeki, 1964). However, it is not known if mutation and inactivation occur by the same mechanism. Cramer (pers. comm.) found that phage T4 inactivated by the photodynamic action of acridine orange can be cross-reactivated, (cross-reactivation, a rescue phenomenon similar to MR, also involves a recombination process). This indicates that inactivation is caused by discrete lesions along the genome which leave the undamaged regions able to participate in genetic recombination with other phage genomes.

Light dose: (min.)	Fraction of infected cells producing plaques:	
	Single infection (m = 0.012)	Multiple infection (m = 2.5)
0	1	1
2	1.8×10^{-1}	5.9×10^{-1}
4	5.6×10^{-3}	2.6×10^{-1}
6	3.2×10^{-4}	9.3×10^{-2}
7	2.4×10^{-5}	3.3×10^{-2}

Table 1. Phage $T4^+$ at 10^{10} particles/ml. was pretreated in darkness for 4 hr. at 37°C. with 4 µg/ml. proflavine. The phages were diluted 10-fold in buffer and irradiated with visible light for 0, 2, 4, 6 and 7 min. Each phage sample was used to infect two aliquots of a log-phase culture of E. coli B at multiplicities (m) of 0.012 and 2.5 phage/cell. Following a 5 min. adsorption period at 37°C. samples were assayed on E. coli B to determine the fraction of infected cells yielding viable phage progeny.

Chemical and physical data from in vitro DNA systems is in agreement with the biological results presented here. Simon and Van Vunakis (1962) found that the photodynamic action of methylene blue on free DNA nucleotides results primarily in the destruction of guanine. Visible light irradiation of salmon testis DNA produces a variety of physical changes which indicate destruction of the DNA, possibly involving single strand scissions (Friefelder, Davison & Geiduschek, 1961).

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