INACTIVATION OF PHAGE T4 BY ETHYLMETHANE SULFONATE

A. Ronen
Laboratory of Genetics
Hebrew University of Jerusalem
Israel

Received August 16, 1968

When bacteriophage T4 is treated with the alkylating agent ethylmethane-sulfonate (EMS), inactivation of the plaque-forming capacity (primary, or immediate inactivation) follows a curve which is initially shouldered but becomes exponential at higher doses. This was interpreted by Brookes and Lawley (1963) as indicating a multi-hit mechanism of killing. However, Krieg (1963) obtained a killing curve that failed to become exponential down to 10^{-6} survival, and interpreted it as indicating a one-hit, two-step mechanism of killing.

Further incubation of the alkylated phage in vitro in the absence of the alkylating agent, is followed by secondary (delayed) inactivation, which results from the hydrolysis of alkylpurines off the DNA (Loveless, 1959; Loveless and Stock, 1959; Bautz and Freese, 1960; Brookes and Lawley, 1963). It was suggested by Loveless (1959) and by Brookes and Lawley (1963) that such hydrolysis might be the only mechanism of inactivation, while no true immediate inactivation results from ethylation as such.

The present communication presents evidence that immediate inactivation of EMS-treated phage takes place as the result of a process other than hydrolysis, which follows essentially one-hit kinetics.

Experimental

Bacteriophage T4 rII 1272 was treated with EMS in a medium containing equal volumes of 0.08M phosphate buffer and Difco nutrient broth, pH6.8 at 43.5°C. Final concentration of the agent was 4% (v/v). Samples were taken every five minutes and diluted at least 1,000-fold in ice-cold nutrient broth, pH6.8. Post-treatment incubation was done in the same medium, at 43.5°. Samples were taken every fifteen minutes and kept on ice. Determination of survival was done at the end of the post-treatment incubation period, by plating the phage on E.coli B in soft agar, incubating the plates at 37° and counting the plaques after twelve to twenty hours.

RESULTS

Phage survival (immediate inactivation) follows a shouldered but exponential curve down to nearly 10^{-7} (heavy, solid line in fig. 1). The data for the lowest part of this curve (treatments of 80 - 100 min.) were taken from two sets of plates: in one set the phage had been adsorbed to the indicator bacteria at a total multiplicity of infection (moi) of 0.001 particles per cell or less (open circles). In the other the moi was 0.1 (closed circles). The results obtained from the two sets of platings are essentially the same, indicating lack of multiplicity reactivation of the EMS-inactivated phage. The lines marked 0 to 100 in fig. 1 represent post-treatment inactivation data, covering a 90 min. period immediately following the dilutions that terminated the treatment. No fall of titer is observed in the control phage (drawn from the treatment tube at zero time after addition of EMS). On the contrary, phages that were subjected to treatments from 5 to 100 min., show increasing rates of post-treatment inactivation with half-life periods falling from several hours down to ca. 20 min.

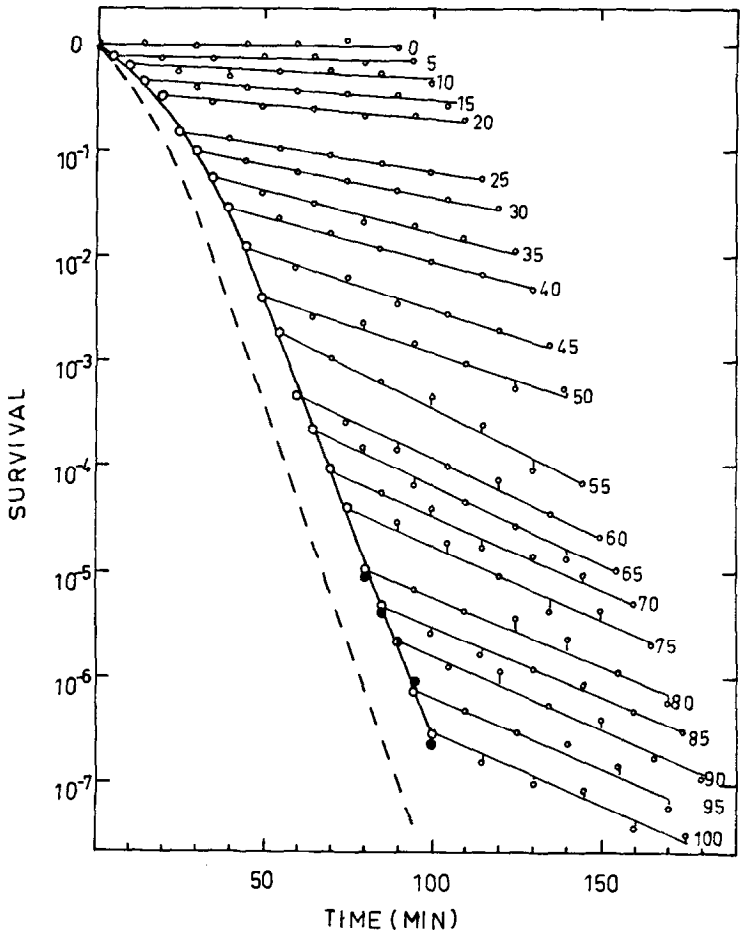

Figure 1. Inactivation of phage T4 by ethylmethane sulfonate. The phage was treated up to 100 min., as described in the text. Primary inactivation is represented by the heavy, solid line (large circles). Each thin line (small circles) represents delayed inactivation of phage that have been treated for the number of minutes denoted by the corresponding figure. The broken line is obtained by connecting the right ends (90 min. of post-treatment) of the delayed-inactivation curves. Open circles represent data from platings at an moi of 0.001 or less. Solid circles represent data from platings at an moi = 0.1.

The rates of inactivation of the alkylated phage upon post-treatment incubation are plotted in fig. 2 against the average numbers of lethal hits received by the phage by the end of the treatment. These rates are calculated as $k = \ln(P/Po)/t$, where P is the phage survival at a given time of post-treatment. Po

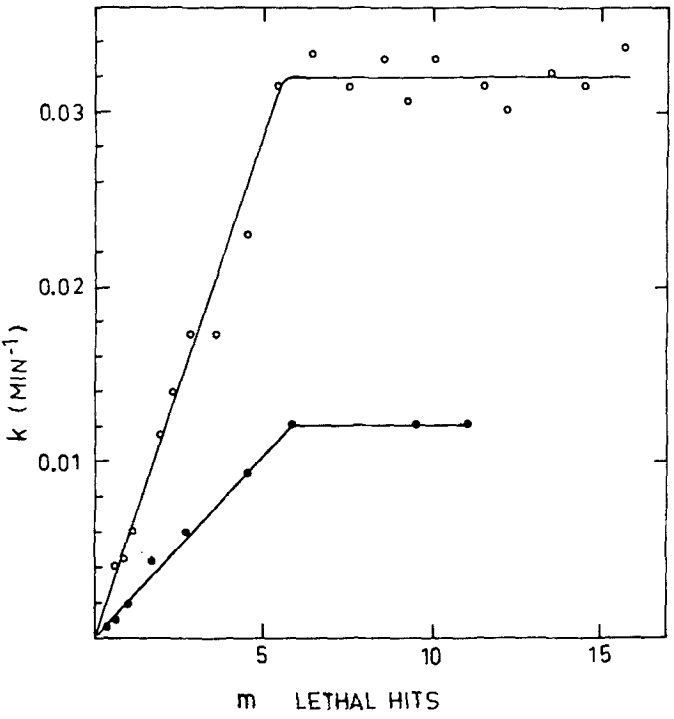

Figure 2. The rate of post treatment inactivation of phage T4 as a function of the number of lethal hits received by it by the end of the treatment. The rate of inactivation is calculated as $k = \ln(P/Po)t$. The number of lethal hits, m, is calculated from the primary-inactivation curve, in fig. 1, where the surviving fraction of the phage population represents the zero term of the Poisson distribution, $P/Po = e^{-m}$.

---

is the survival at zero time, and t is time in minutes. It can be seen (open circles) that k increases linearly until it reaches a maximum value of 0.032 min^{-1} when the phage has sustained an average of 5-6 lethal hits. In another experiment, which was done at 37° (closed circles), k reached a maximum value of 0.012 min^{-1} at about the same level of primary inactivation. This is also shown in fig. 1 by the essentially parallel delayed-inactivation curves for phage treated 55 minutes or longer. These curves correspond to a rate of inactivation seven times lower than that of the primary inactivation.

DISCUSSION

For any level of treatment the immediate inactivation curves in fig. 1 are much steeper than the corresponding part of the delayed inactivation curve. Since the whole experiment was done at the same temperature, this can only be interpreted as indicating that different mechanisms are involved in the two processes, (there are no differences between primary- or delayed-inactivation curves obtained in broth or in buffered broth, the only effect of the buffer being the control on pH in the presence of EMS). One mechanism causes delayed inactivation and involves hydrolysis of 3-ethyladenine and 7-ethylguanine groups off the DNA (Brookes and Lawley, 1963). In the ethylated phage DNA, 3-ethyladenine is roughly ten times less frequent than 7-ethyl-guanine, but its rate of hydrolysis is about six times higher (ibid). The post-treatment inactivation curves therefore represent the complex resultant effects of the two hydrolyses. These effects cannot, however, account for the immediate inactivation (which is, under the present conditions, at least seven times more efficient than the delayed one). This follows from the failure of the delayed-inactivation curves to extra-polate through the origin of the primary killing curve.

Like delayed inactivation, primary inactivation is essentially exponential, which rules out a multi-hit mechanism of killing. A likely explanation for the shoulder observed at short exposures to EMS is the relative ability of the phage to repair small amounts of alkylation-induced damage. The ability of some HCR^+ bacterial strains to repair monofunctional-alkylation damage in phage SP01 has been demonstrated by Strauss and Wahl (1964). In fact, the shoulder in the survival curve of phage

T4 can be made smaller by interference with phage-induced repair processes (unpublished results).

Unlike primary damage, delayed damage cannot be repaired. This follows from the fact that all the corresponding curves in fig. 1 follow straight, shoulderless lines. It is also shown by the broken line in fig. 1, which represents the survival curve after the end of the 90-min. period of post-treatment incubation.

If immediate killing is caused by the mere presence of alkyl groups on the phage-DNA molecule, while delayed inactivation results from breakdown of the alkylated (but hitherto functionally intact) DNA, the plateaus in fig. 2 can be explained as follows: at any temperature of post-treatment incubation, steeper slopes of inactivation result from more extensive alkylation of the phage DNA. This is true, however, only as long as the number of alkyl groups on the DNA molecule stays below some critical level, which is the number of alkylations required to produce a "lethal hit". This number was shown (Brookes and Lawley, 1963) to be roughly 800. This necessarily puts an upper limit to the rate of post-treatment inactivation that may ensue, as only phages with less than the critical number of alkylations are left for further inactivation. The mechanism through which alkylation per se may cause the phage to lose its infectivity is still obscure. The absence of multiplicity reactivation of the EMS-treated phages, both in the present case and in phage λ (unpublished results), indicates that both types of inactivation may result from failure to carry on some function(s) prior to DNA replication.

REFERENCES

Bautz, E. and E. Freese, 1960, Proc.Nat.Acad.Sci. 46:1585.
Brookes, P. and P.D. Lawley, 1963, Biochem. J. 89:138.
Loveless, A., 1959, Proc. Roy. Soc. B.150:497.
Loveless, A. and J.C. Stock, 1959, Proc. Roy. Soc. B.150:423.
Krieg, D., 1963, Genetics 48:561.
Strauss, B.S. and R. Wahl, 1964, Biochim. Biophys. Acta. 80:116.