HOST-CELL REACTIVATION OF ULTRAVIOLET-DAMAGED PHAGE IN STREPTOCOCCUS PYOGENES

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The term "host-cell reactivation" (HCR) denotes an enzymatic light-independent reaction which removes lethal damages induced by ultraviolet light (UV) in bacterial DNA or in the DNA of some species of infecting phage (Rupert and Harm, 1966). The excision of pyrimidine dimers appears to be a part of the repair process, for it occurs in wild type E. coli, but not in certain mutants which display a great increase in the sensitivity to the lethal effects of UV and a decrease in the number of plaques formed by UV-irradiated phage that may infect these cells (Howard-Flanders et al., 1966). From studies with phage or infectious DNA it is concluded that HCR is present in E. coli, S. typhimurium, M. lysodeikticus, S. marcescens, and H. influenzae (Rupert and Harm, 1966). Since photobiological and genetic studies are usually made with a selected number of strains derived from a very limited variety of bacterial species little can be said about the occurrence of HCR in bacteria in general.

In a previous paper (Malke, 1967b) it has been shown that caffeine and acriflavine (which are known to block HCR; Metzger, 1964; Sauerbier, 1964; Feiner and Hill, 1963) enhance the killing and prophage inducing effects of UV in a lysogenic strain of Streptococcus pyogenes, suggesting that this species is also able to perform dark repair of UV-damaged DNA. This paper presents conclusive evidence in support of this suggestion in that it describes the isolation and some properties of mutants that have lost the capacity to reactivate UV-inactivated phage.

MATERIALS AND METHODS

Strains - Str. pyogenes strain K 56 was used throughout. It is a group A, type 12 strain (Kjems, 1958) which appears to be non-lysogenic. The clear plaque-forming mutant c 3 of the temperate streptococcal phage P 7738 (Malke, 1967a) was used for this study. It has been isolated by mutagenesis of P 7738 with 1 M hydroxylamine according to Freese et al. (1961). It has, if any, a strongly reduced capacity to lysogenize strain K 56.

Media - Serum broth (SB) consisted of 10 g proteose peptone No. 2 (Difco), 3 g NaCl, 2 g $Na_2HPO_4 \cdot 12\ H_2O$, 0.2 g $CaCl_2$, 1 g glucose, 1000 ml beef heart broth, and 10 per cent horse serum; pH 7.2. With the addition of 2 per cent agar, serum agar (SA) contained the same constituents as SB but the amounts of beef heart broth and serum were reduced to 20 and 2 per cent, respectively. To the soft agar (0.7 per cent agar) serum was added to a concentration of 20 per cent, whereas beef heart broth was omitted. For irradiation, bacteria and phage were suspended in buffer of the following composition: 7 g $Na_2HPO_4 \cdot 12\ H_2O$, 3 g KH_2PO_4, 4 g NaCl, dissolved in 1000 ml aqua dest.; 4 ml of 0.5 M $MgSO_4$ solution was added after autoclaving.

UV-irradiation - Stationary phase cells grown in SB and, after washing in buffer, diluted to a titer of 10^6 colony forming units/ml were exposed to irradiation from a low pressure mercury vapor lamp (BGW, S 375). Irradiation was carried out at two fixed distances in petri dishes in which the liquid level did not exceed 1.0 mm. Phage to be irradiated was grown on strain K 56 and diluted to give 10^6 plaque forming units/ml. After suitable dilution irradiated bacterial suspensions were plated in quadruplicate on SA. Phage P 7738 c 3 was plated with 0.1 ml of log phase bacteria in surface technique without preadsorption. To prevent photoreactivation, all irradiation and subsequent handling was performed under subdued light.

Isolation of Hcr^- mutants - Hcr^- mutants of strain K 56 were isolated according to the procedure described by Howard-Flanders and Theriot (1962). A series of tubes containing 10^8 colony forming units/ml were treated with

100 ug/ml of N-methyl-N'-nitro-N-nitrosoguanidine in phosphate buffer at pH 7.0 for 30 min at 37°C. After washing the cells were diluted 1 : 4 in fresh SB and regrown overnight. After plating for viable units the cultures were stored overnight in the refrigerator. Between 500 and 1000 colony formers were then plated on SA and after 4 hours at 37°C a suspension of phage P 7738 c 3, previously irradiated with UV (N/N_0 = 10^{-3} to 5.10^{-3}), was gently poured over the bacteria. 7.10^8 irradiated phages were added to each plate. After further incubation this treatment gave rise to 156 colonies on 40 plates from the 30,000 treated bacteria plated. Of the 108 colonies picked for study 57 could not be distinguished from the wild type when tested with the semi-quantitative method outlined by Greenberg (1967), 35 were phage resistant, 12 (which might be siblings) were of intermediate sensitivity, and 4 were very sensitive to UV. The strains with which this paper is concerned were of independent mutational origin.

Although this method has originally been described for a virulent phage as a selective agent it is also applicable to temperate phage if only mutants are available which lysogenize poorly or not at all.

Chemicals - N-methyl-N'-nitro-N-nitrosoguanidine, prepared by Dr. P. Neuland of this institute; caffeine (Arzneimittelwerk Dresden); acriflavine (Bayer Leverkusen).

RESULTS AND DISCUSSION

The properties of the wild type and of five Hcr$^-$ mutants as regards sensitivity to UV were examined by measuring the survival of UV-irradiated phage P 7738 c 3 when plated on each strain (Fig. 1). While it is apparent that unirradiated phage give identical plaque counts using either strain as plating indicator the slope and shape of the survival curve of the irradiated virus is strongly influenced by the host used for assay. The survival curve of the phage, plated on the wild type, shows a marked multihit-like shoulder, indicating that at low doses, reactivation is very effective. The four most sensitive mutants as regards ability to support growth of UV-inactivated phage fall into a single class with identical properties. The fifth, however, has retained considerable residual HCR activity. The host-cell reactivable

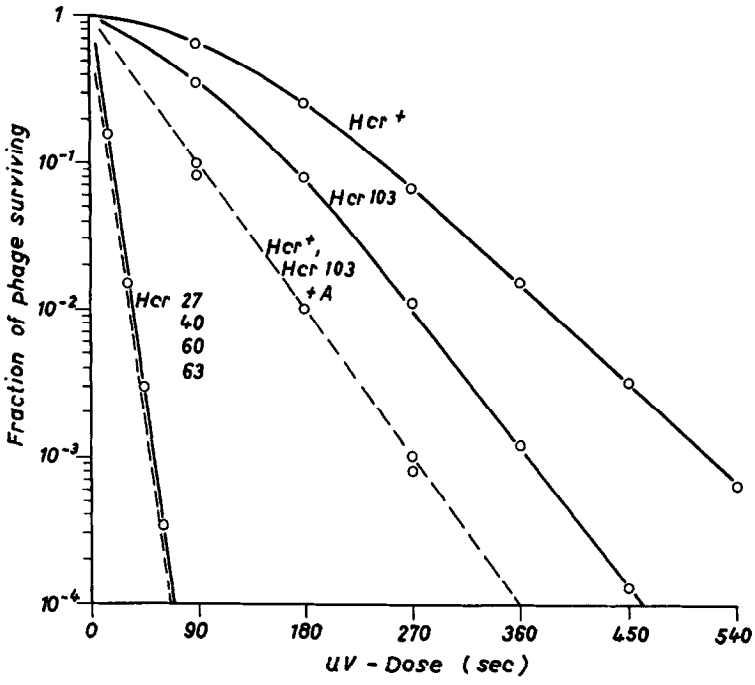

Fig. 1. Survival of phage P 7738 c 3 on Streptococcus pyogenes K 56 Hcr^+ strain and on five Hcr- mutants of independent origin. Broken lines: 0.1 µg/ml of acriflavine (A) in the plating medium.

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sector as calculated from the straight parts of the phage survival curves on K 56 wild type and the most UV-sensitive mutants is 0.9.

It was of some interest to know wether or not UV-enhancers such as caffeine and acriflavine could further cut down phage survival in the mutant strains. Caffeine was incorporated in the post-irradiation agar medium at a concentration of 2 mg/ml. This concentration decreases the efficiency of plating of unirradiated phage on wild type to 2.10^{-1}, and the HCR capacity is only slightly interferred with. Thus, caffeine seems to be unsuitable for inhibition experiments in the system studied. Acriflavine at a concentration of 1 µg/ml completely inhibits growth of the plating indicator. A concentration of 0.1 µg/ml allows growth to take place and has no effect on viability of phage in the controls. At this concentration acriflavine reduces the survival of UV-irradiated phage when plated on the wild type and on the

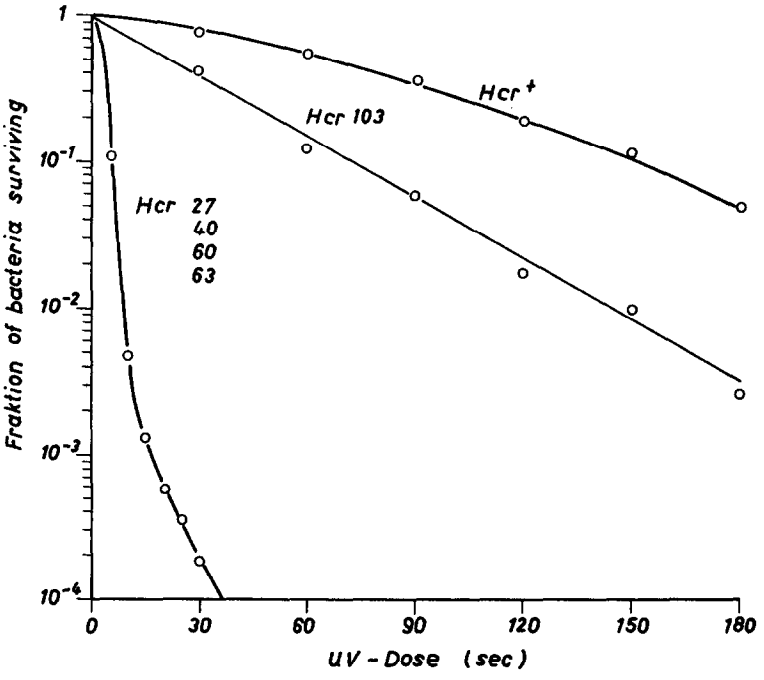

Fig. 2. Survival of the colony forming ability of Streptococcus pyogenes K 56 Hor^{+} and of five Hcr^{-} mutants of independent origin.

mutant of intermediate sensitivity but the inhibition of HCR is evidently rather incomplete (approximately 40 per cent). Phage survival on the most UV-sensitive mutants cannot be influenced by acriflavine, so these appear to be nonleaky.

Response of the colony forming ability of the bacteria to UV-irradiation was also tested with the results shown in Fig. 2. In accord with the current idea that failure to reactivate UV-induced lesions in phage is accompanied by a high UV-sensitivity of the cell itself, the response pattern for UV of the bacterial strains used here is obviously similar to that observed for phage grown on them. The survival curve of the class of the most sensitive mutants shows a pronounced tailing at higher doses, probably due to the presence in the population of a more resistant component.

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