ON THE DARKREACTIVATION MECHANISM IN ULTRAVIOLET IRRADIATED BACTERIA

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The decrease in the rate of photoreactivation (PhR) of ultraviolet (u.v.) irradiated phage due to u.v. irradiation of bacteria (Lennox et al. 1954) is understood as a competing effect of the u.v. lesions in the bacterial nucleic acids for the PhR enzyme (Metzger 1963). This competing effect is neutralized either by darkreactivation (DR) or by PhR of the bacteria before phage infection. The occurrence of DR is restricted to bacteria which can perform hostcell-reactivation (HCR) on u.v. irradiated phage T1. For HCR of phage T1, Sauerbier (1961, 1962a, 1962b) has proposed an enzymatic repair mechanism. The strictly linked occurrence of HCR and DR suggests that the two phenomena are based on the same mechanism. The experiments described in this paper demonstrate: 1) HCR and DR are achieved by the same enzyme. 2) DR directly repairs the u.v. lesions. 3) PhR and DR repair the same lesions in nucleic acids. 4) The enzyme is neither induced by phage infection nor by u.v. irradiation, but is normally present in the bacterial cell. 5) The action of the enzyme is inhibited by caffeine.

MATERIALS AND METHODS

The bacterial strains used: Escherichia coli B 94 (deficient for arginine and adenine, Gollub and Gots 1958), $B_{s-1}hcr^-$ (Hill 1958), K12S hcr^- (Harm 1963), and K12S hcr^+. E. coli B 94 was grown to a concentration of $4x10^8$/ml in M-9 medium supplemented with 10^{-3} M

arginine and 80 µg/ml adenine. The cells were washed and resuspended at a concentration of $1x10^6$/ml in M-9 without adenine, starved for one hour for adenine and then irradiated at room temperature, using a low pressure mercury lamp (Osram HNS 12), which emitted predominantly the wavelength 2537 Å. After irradiation, the bacteria were either directly plated or incubated at 37° in M-9 without adenine and plated at the indicated times on caffeine agar (concentration in the bottom agar 0.1%, and in the top layer agar 0,6%). In some experiments subsequent to the starvation for adenine, the cells were washed three more times, resuspended in M-9, starved one more hour for arginine and then irradiated. In this latter case, the bacteria were incubated after the irradiation in M-9 without adenine and arginine. Irradiated and unirradiated controls were plated after the same incubation times on tryptone agar without caffeine. Adsorption of phage was done for 20 min at 37° in phosphate buffer containing $2,5x10^{-3}$M Mg SO_4 (concentration of bacteria $1x10^9$/ml, multiplicity of infection $1x10^{-3}$), and free phage were removed by centrifugation. In the experiments in which the phage-bacterial complexes were plated on caffeine agar, the adsorption, resuspension, and dilution were done in the presence of 0,25% caffeine.

RESULTS

It was concluded from our results, and the results of Lieb (1961) that caffeine inhibits DR which very likely is identical with HCR (Metzger 1963). To test this conclusion, u.v. irradiated phage were plated on agar with and without caffeine using as plating bacteria: 1) *E.coli* K12S hcr^+ and, 2) the non-hostcell reactivating mutant K12S hcr^- (Fig.1). Parallel to this, samples of the bacteria used as host bacteria for the phage were u.v. irradiated, and the colony-forming ability was measured by plating them on agar with and without caffeine. (Fig.2). The efficiency of plating of unirra-

diated phage and the colony-forming ability of unirradiated bacteria are not influenced by the concentration of caffeine used. U-gene reactivation (Harm 1959) of u.v. irradiated phage T4, PhR of u.v. irradiated phage and bacteria E.coli B, and multiplicity reactivation of phage T2 do occur in the presence of caffeine.

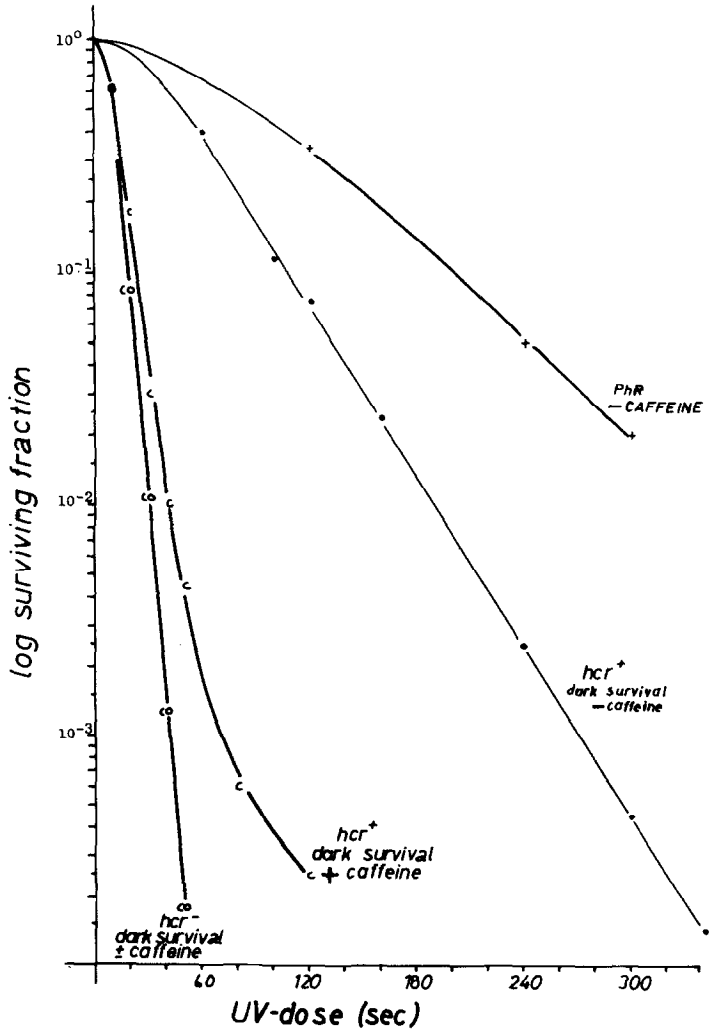

Fig.1. Inhibition of hostcell reactivation of phage by caffeine. a)On host bacteria E.coli K12S hcr⁺: Darksurvival on tryptone agar; caffeine absent. Survival after PhR in liquid medium and plating on tryptone agar; caffeine absent. Darksurvival on caffeine agar. b)On K12S hcr⁻: On tryptone agar with and without caffeine.

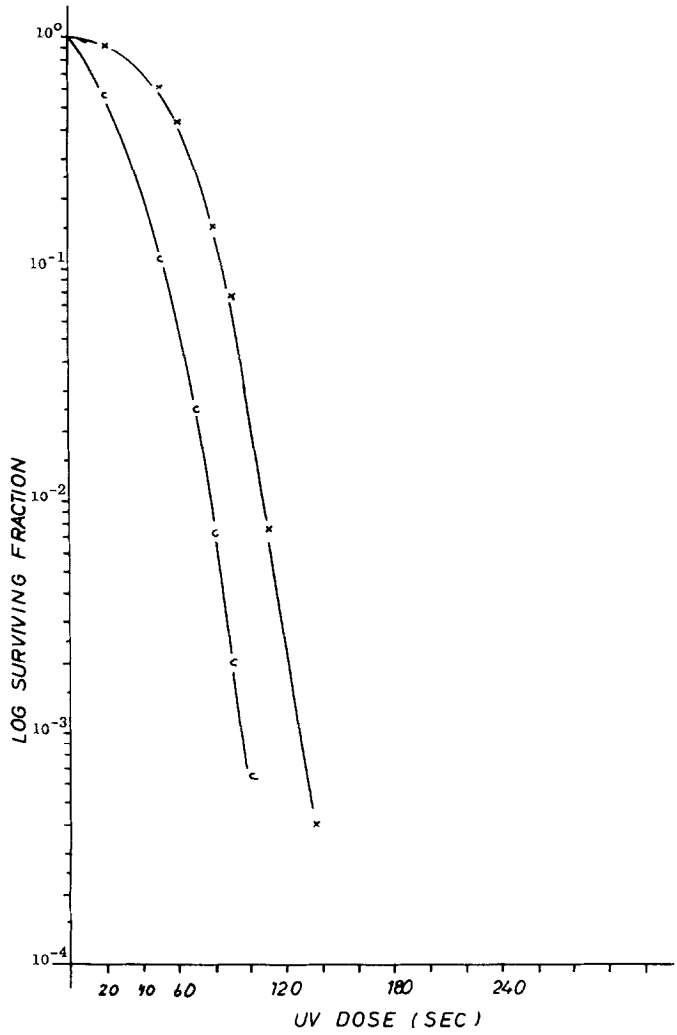

Fig.2. Inhibition of darkreactivation of E.coli K12S hcr$^+$ by caffeine.
x U.v. survival after plating on tryptone agar.
c U.v. survival after plating on caffeine agar.
U.v. dose of one second is equivalent to about seven erg/mm^2.

As can be seen from the figures, there is little difference between the u.v. sensitivity of phage on the hostcell reactivating wild-type K12S hcr$^+$ when plated on caffeine agar and that on the non-hostcell reactivating mutant hcr$^-$, both on normal and on caffeine agar. The same results are obtained with phage T1 (see Sauerbier 1964). This demonstrates, as we had concluded from our earlier ex-

periments, that caffeine does inhibit the DR mechanism. As observed with phage, the u.v. sensitivity of hostcell reactivating bacteria E.coli B, B/r, and K12S hcr^+, but not that of the non-hostcell reactivating mutant $B_{s-1}hcr^-$, is increased when plated on caffeine containing agar (Fig.2, Fig.3). Bacillus subtilis had also an increased u.v. sensitivity when plated on caffeine containing agar, indicating that DR of u.v. lesions does occur in this bacterium. Due to this inhibitory effect, caffeine could become a suitable tool in transformation experiments where inhibition of DR is necessary. Fig. 3 shows the results obtained with E.coli B 94 when plated with and without caffeine. U.v. irradiation results in an inactivation curve with two different slopes representing two different fractions of bacteria. The sensitivity of the fraction representing about 80% of the bacteria - i.e. the first part of the curve - is eight fold increased when bacteria are plated on caffeine agar, whereas the sensitivity of the 20% fraction - i.e. the second part of the curve - remains unchanged. However, the fraction decreases from 20 % to about 0.4%.

If DR occurred in the absence of DNA and RNA synthesis, it would demonstrate that DR works by direct repair. To test this, u.v. irradiated B 94 (starved for adenine, see Volkin 1960) was incubated in M-9 without adenine for different times and then plated with and without caffeine. The curves in Fig. 4 show that the survival after u.v. irradiation icreased with incubation time in an adenine deficient M-9 medium and subsequent plating on caffeine agar. The final survival was the same as if they were plated immediately on agar without caffeine. Unirradiated controls did not divide during the time the reactivation occurred. Thus, it is demonstrated that the DR directly repairs the u.v. lesions in the bacterial nucleic acids. To prove that the DR enzyme is present in the bacteria nor-

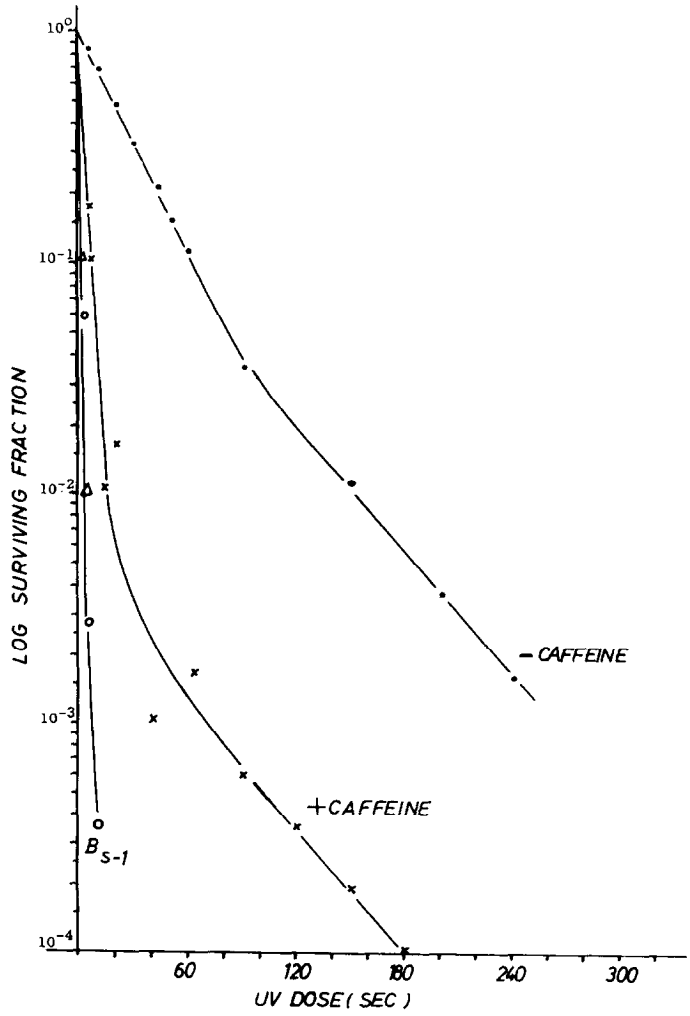

Fig.3. Inhibition of darkreactivation of E.coli B_{s-1} and B94 by caffeine.

B_{s-1} on tryptone agar. B_{s-1} on caffeine agar. B94 on tryptone agar. B94 on caffeine agar. U.v. dose of eight seconds is equivalent to about seven erg/mm^2.

mally, the same experiments were done with E.coli B 94 starved for adenine, and in addition to that, starved one more hour for adenine and arginine, and then irradiated. DR occurred as if the bacteria had not been starved for arginine. Thus, the DR enzyme was present before irradiation. To test the assumption that the DR enzyme and

the PhR enzyme can repair the same lesions in the DNA, u.v. irradiated phage were adsorbed to K12S hcr$^+$ in the presence of caffeine, photoreactivated in the presence of caffeine and plated on caffeine agar. The curve obtained is identical with the inactivation curve obtained after plating on caffeinefree agar in the dark (see Fig.1). This demonstrates an overlapping action of DR and PhR.

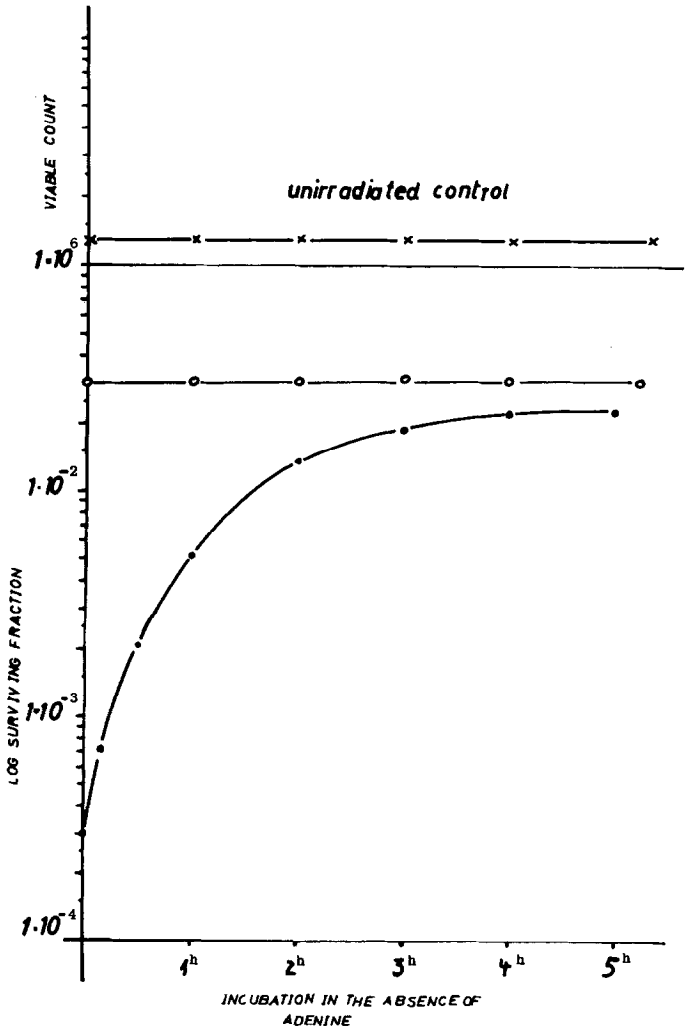

Fig.4. Darkreactivation of u.v. irradiated E.coli B94 under conditions precluding synthesis of nucleic acids.

Bacteria were starved for one hour in adeninefree M-9, washed, resuspended in M-9 without adenine and irradiated at a concentration of $1x10^6$/ml. Then the cells were incubated in adeninefree M-9 and plated after the indicated times on agar containing caffeine.

Irradiated bacteria on agar without caffeine.

Unirradiated control on agar containing caffeine.

DISCUSSION

Experiments of Sauerbier (1961, 1962a, 1962b) demonstrate that it is very probable that the HCR of u.v. irradiated phage is an enzymatic repair mechanism. The discovery of mutants which: 1) can not perform HCR on phage and 2) have an increased u.v. sensitivity (Hill 1958, Howard-Flanders 1962, Harm 1963, Rörsch et al. 1963) and 3) our experiments, in which the competition of u.v. lesions in bacterial nucleic acids for the PhR enzyme was neutralized upon DR of the bacteria (Metzger 1963), support the hypothesis that an enzymatic DR mechanism repairs u.v. damages in phage and bacteria. It was concluded from our work that the DR enzyme is not induced by u.v. irradiation or by phage, and strong evidence was given that it directly repairs u.v. lesions in bacterial DNA.

HCR of u.v. lesions in the DNA of phage and DR of u.v. lesions in bacterial DNA are prevented by caffeine. On the other hand, caffeine does not prevent the reactivation of u.v. lesions in phage DNA by enzymatical mechanisms like PhR or u-gene reactivation. Furthermore, the efficiency of plating of unirradiated phage and bacteria and multiplicity reactivation of u.v. irradiated phage T2 are not influenced by caffeine. These results together with the findings summarized in the introduction demonstrate that HCR and DR are based on the same mechanism.

The inhibition by caffeine of DR of u.v. lesions in phage and bacteria enabled us to do experiments giving evidence for the direct repair of u.v. lesions by DR. For these experiments we used bacteria which are deficient for arginine and adenine. DR of the u.v. irradiated bacteria was unchanged in synthetic medium in the absence of adenine, i.e. without synthesis of nucleic acids. Therefore this demonstrates that the DR directly repairs the u.v. lesions.

DR was also not influenced if the experiments were done with bacteria starved for adenine and arginine. This demonstrates that the

enzyme was present in the cell before starvation, since under these conditions protein synthesis was blocked. The PhR of u.v. irradiated phage is possible in the presence of caffeine. The survival after PhR in caffeine containing medium is identical with the dark survival curve which is obtained by plating her^+ host bacteria in the absence of caffeine. Thus, the PhR enzyme repairs lesions which are otherwise repaired by DR. The action of the caffeine molecule is not known. Since the PhR enzyme can reactivate u.v. lesions in the presence of caffeine, the caffeine molecule does not seem to combine with the u.v. lesion in the DNA. It is more likely that it interacts with the DR enzyme directly.

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