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PHOTOREACTIVATION OF UV-DAMAGE IN PHAGE CONTAINING 5-BROMOURACIL-DNA

By

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With 1 Figure in the Text

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Introduction

Great interest has been attracted by the observation that photoreactivation (PHR) (STAHL *et al.*, 1961) and host-cell reactivation (HCR) (SAUERBIER, 1961) are blocked in UV-damaged phage heavily substituted with 5-bromouracil (BU). It has been clearly demonstrated that the reactivable sector decreases and UV-sensitivity increases with increasing amounts of incorporated 5-bromouracil. These findings led to the widely accepted view that substitution of thymine by BU renders the DNA more UV-sensitive largely by virtue of the irreversibility of the damage. Recently, however, we found that compounds of the cysteine-cysteamine group, if present during irradiation, abolish the sensitizing effect of this base analogue in phage T1 and bring back HCR to its normal amount (HOTZ, 1963, HOTZ and ZIMMER, 1963).

Concurrent studies on the disappearance of thymine dimers from UV-irradiated DNA by excision of the damaged part (SETLOW and CARRIER, 1964) encouraged us to examine the specifity of HCR and of PHR with respect to the class of photoproducts removed. A high degree of specifity was indicated by the observation that substitution of 5-BU for thymine blocks the known recovery processes. Reactivation of 5-BU-photoproducts similar in amount to that occuring in the thymine-phage both in the case of PHR and of HCR, however, could be considered to be more in favour of excision-like mechanisms to occur for both HCR and PHR. This communication deals with the UV-protective action of cysteamine on phage T1 the DNA of which has been heavily substituted by 5-BU, and with the influence of this substitution on photoreactivation of the phage.

Materials and Methods

The technique of incorporating 5-bromodeoxyuridine in phage followed a method published by STAHL *et al.* (1961). Strains of phage, assay of the plaque-forming ability, irradiation technique, as well as density measurement by CsCl-gradient centrifugation were described previously (HOTZ, 1963, HOTZ and ZIMMER, 1963). *E. coli* B_{s-1}, kindly supplied by Dr. W. HARM, Cologne and originally isolated by Dr. RUTH F. HILL, New York, were used for plating. This strain does not perform HCR.

Results

Fig. 1 shows the results of comparative experiments with normal and substituted 5BU-T1 suspended in buffer and UV-irradiated in the presence and absence of cysteamine. From the dose-survival curves it is evident that:

1. The sensitizing effect due to BU-incorporation is observed when phage are plated in the dark on a bacterium lacking HCR. Though the difference in final slope of the survival curves of normal (curve Th) and substituted phage (curve BU) is small, a complete elimination of the initial shoulder is found with 5BU-T1. This can not be due to an effect of residual HCR-ability in strain B_{s-1} since the curves Th and curve BU are identical to those obtained after adsorption on heavily UV-irradiated *E. coli B* (HOTZ, 1964).

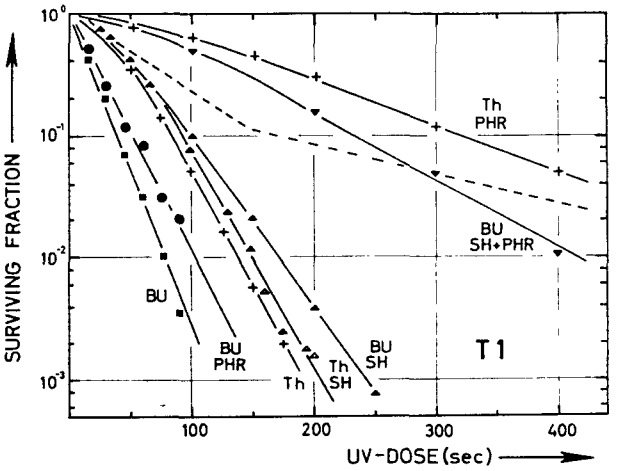

Fig. 1. Surviving fraction of buffersuspended T1 phage after UV-irradiation and adsorption on *E. coli* B_{s-1}. (PHR) indicates maximum photoreactivation, otherwise the experiments are performed in the dark. (SH) indicates irradiation is done in the presence of M/100 cysteamine. (BU) indicates 5 BU-phage and (Th) normal control T1. The dashed line represents, for comparison, T1 wildtype survival after plating on HCR-positive strain *E. coli B*

2. Photoreactivation is reduced to a small sector in 5BU-T1 (curve BU, PHR). This small reactivable sector is probably due to incomplete substitution.

3. The presence of M/100 cysteamine during irradiation does not alter the survival rate of normal phage (curve Th, SH). However, this compound is able to remove effectively the radiosensitization in 5BU-phage (curve BU, SH).

4. Photoreactivation is nearly normal (curve Th, PHR) if the substituted T1 is irradiated in the presence of M/100 cysteamine (curve BU, SH + PHR).

Discussion

Evidence from photochemical and radiochemical studies indicates that irradiation of 5-halogen uracil results in dehalogenation leaving a uracil-like structure (WACKER, 1963, SMITH, 1963, LOCHMANN, 1963). It has been concluded (HOTZ, 1963) that "desensitization" of 5BU-DNA by sulfhydryls is due to a reaction between the protective substance and the irradiated 5-bromouracil preventing destruction of the pyrimidine ring after dehalogenation. This reaction was found to be possible in wet but not in dry systems, a result suggesting differences in the mechanisms of protection from 5BU-damage and of the well-known protective effect of sulfhydryls on *normal* phage inactivated by *ionizing* radiation. The latter type of protection was found to occur in the wet as well as in the dry state, and even at liquid nitrogen temperature, and has been discussed recently

in detail (HOTZ and ZIMMER, 1963). Our results concerning protection against UV-damage due to 5BU-incorporation further led us to assume that photoproducts of UV-damage in halogen-base substituted phage differ qualitatively from those produced in "ordinary" UV-damage, otherwise one would expect UV-irradiated *normal* phage also to be protected by cysteamine. Such protection, however, was not observed in our experiments. The results and the conclusions concerning SH-protection against UV-damage in BU-DNA suggest that the —SH compounds prevent some secondary chemical reactions resulting in a completely altered DNA-structure which can not be restituted by the host-cell. As regards the kind of radiation damage due to 5BU-reactions occurring in the absence of cysteamine an observation on phage Lambda (FOX and MESELSON, 1963) may be of particular interest. In this experiment a "hybrid" phage was used, with 5-BU substituted for thymine in just one of the two DNA strands. This hybrid was irradiated by "visible light" from a fluorescent lamp in the presence of AET (2-aminoethyl-isothiouronium bromide), which belongs to the cysteine-cysteamine group and has a radioprotective action similar to that of cysteamine. Under these conditions the heavy strand only was damaged, since at the wavelength employed the energy absorbed in the thymine strand could be neglected. In the absence of sulfhydryl, however, both strands appeared to be damaged (MESELSON, personal communication) probably because of more extensive reactions of 5-BU leading to lesions of the sister strand. These results suggest that one of the main functions of cysteamine is restriction of the 5BU-damage to the strand containing the particular 5 BU-residue hit by the photon.

Two alternative explanations could be given for the observation. that both HCR and PHR occur in 5BU-phage to a normal amount if irradiation is done in the presence of cysteamine:

(i) Intrastrand thymine dimers are a *specific* substrate for reactivating enzymes (SETLOW and CARRIER, 1964, WULFF and RUPERT. 1962, HARM, 1963), but the photoproducts of 5-BU formed in the presence of —SH compounds are removed or split off by these enzymes with similar the same efficiency.

(ii) Reactivation mechanisms of radiation damage are rather *unspecific*.

Explanation (i) is unlikely since the photoproducts of thymine and of BU, UV-irradiated in the presence of cysteamine, are completely different. The most likely mechanism by which recovery processes could handle photoproducts of thymine as well as photoproducts of 5-bromouracil, irradiated in the presence of cysteamine, would be "excision" of an oligonucleotide containing the radiation lesion.

Zusammenfassung

Es wird eine neue Strahlenschutzwirkung des Cysteamins gegenüber photoreaktivierbaren und nicht-photoreaktivierbaren UV-Schäden bei 5-Bromuracil-substituierten Phagen beschrieben. BU-T1 Phagen UV-bestrahlt in Anwesenheit von 0,01 molar Cysteamin zeigen eine normale Strahlenempfindlichkeit und nahezu normale Photoreaktivierbarkeit, die bei Abwesenheit des Schutzstoffes blockiert ist. Der Mechanismus der Desensibilisierung wird diskutiert.

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