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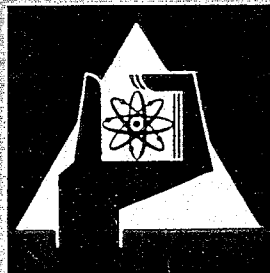
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Zusammenfassung. Es wird beim Phagen T4*Boʳ* eine Schutzwirkung von Cysteamin gegenüber den nach Einbau von 5-Bromuracil in die DNS biologisch zusätzlich beobachtbaren UV-Strahlenschäden beschrieben. Bei BU-Phagen, nicht aber bei normalen Phagen und auch nicht bei BU-Phagen bestrahlt in Anwesenheit von 10^{-2} m Cysteamin, wird eine Zerstörung von Desoxyribose in der DNS gemessen. Der Mechanismus, der zum Abbau von DNS-Pentose führt bzw. die Reaktionen, die in Anwesenheit eines Radikalfängers den Schutzeffekt bewirken, werden im Rahmen einer Arbeitshypothese diskutiert. Da bei den T-Phagen die Gruppe der näher untersuchten enzymatischen Reaktivierungsmechanismen, nämlich Photo-, Wirtszell- und *u*-Gen-Reaktivierung, hinsichtlich des BU-Phänomens gemeinsame Merkmale zeigt, d.h. Blockierung durch BU und Aufhebung des Blocks bei Bestrahlung in Anwesenheit von Cysteamin, liegt die Annahme nahe, daß Schäden an der Desoxyribose der DNS-Helix für die Blockierung der enzymatischen Reaktivierungsschritte verantwortlich sind.

Introduction

The incorporation of halogenated base analogues such as 5-bromouracil (BU) into the DNA has been found to increase the radiosensitivity of phage (Stahl et al., 1961). Furthermore it was observed that photoreactivation, *u*-gene action (Stahl et al., 1961) and host-cell reactivation (Sauerbier, 1961) are blocked in uv-damaged BU-phage. From these findings the conclusion was drawn that incorporation of BU led to an irreversible radiation damage. Recently, however, it was found that compounds of the cysteine-cysteamine group abolish the sensitizing effect of BU in T1 (Hotz, 1963) — as well as in T-even phage (Hotz, 1966) — and restore host-cell reactivation (HCR) (Hotz and Zimmer, 1963) as well as photoreactivation (PHR) (Hotz, 1964) nearly to the amount observed in normal T1-phage. These results gave the first indication that the best known product of uv-irradiation, i.e. the thymine dimer, could not be the principal site of action for the repair mechanisms working in HCR (Setlow and Carrier, 1964) and in PHR (Wulff and Rupert, 1962) because in BU-DNA thymine dimer can not be formed. Our conclusion was (Hotz, 1964) that the reactivating mechanisms of uv-lesions mentioned are rather *unspecific* and have a broad action spectrum. This view has been supported by evidence for excisionlike mechanisms acting on a variety of different DNA-lesions caused by ionizing radiation (Sauerbier, 1964a) as well as by action of sulfur mustard (Papirmeister and Davison, 1964) mitomycin (Boyce and Howard-Flanders, 1964) and HN2[1] (Hanawalt and Haynes, 1965).

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[1] Abbreviation used: HN2, nitrogen mustard.

How does the protective action of sulfur containing compounds fit into the picture ? Photochemical studies in BU-DNA did not give evidence for any influence of cysteamine on the destruction of 5-bromouracil (Lion, 1965). If the photoproducts of BU are handled by reactivating enzymes with the same efficiency as the photoproducts of thymine when irradiation is performed in the presence of cysteamine, the block of reactivation in a BU-phage in the absence of cysteamine could be suspected to be a lesion at the sugar-phosphate backbone of the DNA. Furthermore, the previously known fact that the major photoproduct of BU is uracil (U) (Wacker, 1963; Smith, 1964) led us to hypothesize that the H-atom necessary for the formation of uracil would originate from the deoxyribose resulting in an alteration at this molecule. These considerations encouraged us to examine the effect of radiation on DNA-deoxyribose.

In the present communication it will be shown for phage T4 Bo^r that in fact damage to the DNA-pentose follows uv-irradiation of BU-phage, a lesion which can be avoided by irradiation in the presence of cysteamine. There is no effect of uv on the deoxyribose of normal phage. A strain of T4 was chosen because with this phage it is possible to test the influence of protective compounds on u-gene reactivation blocked by BU-incorporation. Following infection the u-allele is supposed to produce an enzyme which can repair uv-damaged phage-DNA. This gene renders T4 half as sensitive as T2 (Streisinger, 1956; Harm, 1958).

Materials and Methods

Phage T4 Bo^r, an osmotic-shock resistant mutant of T4 wildtype was used throughout the experiments and a stock of this phage together with the host $E. coli$ B was kindly supplied by Dr. W. Harm (Dallas, USA). The technique of incorporating nonradioactive 5-bromouracil (Calbiochem, USA) as well as ^{14}C-labelled 5-bromouracil (Gesellschaft für Kernforschung, Karlsruhe, Germany; spec. activity 10 μCi/mg; 20 μg/ml growth medium) in phage followed a method published by Stahl et al. (1961). About 90% of thymine was substituted by bromouracil as was determined by chromatography. Plating of phage was done by the standard top layer technique (Adams, 1950) avoiding the occurence of photoreactivation. Prior to irradiation crude lysates were purified by digestion with DNase (20 μg/ml; Worthington, USA) and differential centrifugation. Irradiation technique used in the radiobiological experiments were described previously (Hotz and Zimmer, 1963). For the chemical experiments uv-irradiations were carried out with a specially designed low-pressure mercury-vapor lamp, emitting radiation of 2537 Å mainly (Gräntzel, Karlsruhe; Vycor-glass). The effective dose rate delivered to the phage particles (phage concentration $8 \cdot 10^{10}$/ml M/15 phosphate buffer) was between 20 and 100 ergs/mm^2/sec depending on the particular preparation. It was calculated by using the inactivation dose for phage T4 as a reference (D 1% 230 ergs/mm^2; Setlow and Boyce, 1963). Deoxyribose analysis of phage DNA was carried out after irradiation of the phage particles by a modified diphenylamine reagent increasing the sensitivity of the assay by a factor of about 4 relative to the original Dische procedure (Dische, 1955). Details on this method will be given in a separate paper (Reuschl, manuscript in preparation). It should be mentioned that the deoxyribose as determined by the method of Dische concerns essentially the purine-bound deoxyribose. Analysis for uv-destruction of bromouracil and formation of uracil in ^{14}C-BU-labelled phage was carried out using thin layer chromatography on silica gel (solvent: ethylacetate/isopropanol 65% = 75/25). Phage were hydrolysed before in HCOOH (95%) at 175° C for 35 min. The radioactivity of the products separated by chromatography was counted by a "Berthold" thin layer scanner (Berthold, Wildbad, Germany).

Results

1. Inactivation of the Plaque Forming Ability of UV-irradiated Phage

Fig. 1 shows the results of experiments with normal T4 Bo^r (Th) and 5-bromouracil substituted phage (BU) diluted hundredfold in M/15 phosphate buffer and irradiated in the presence and absence of cysteamine-base (pH 7.2; Fluka, Switzerland). From the dose-survival curves it is evident that:
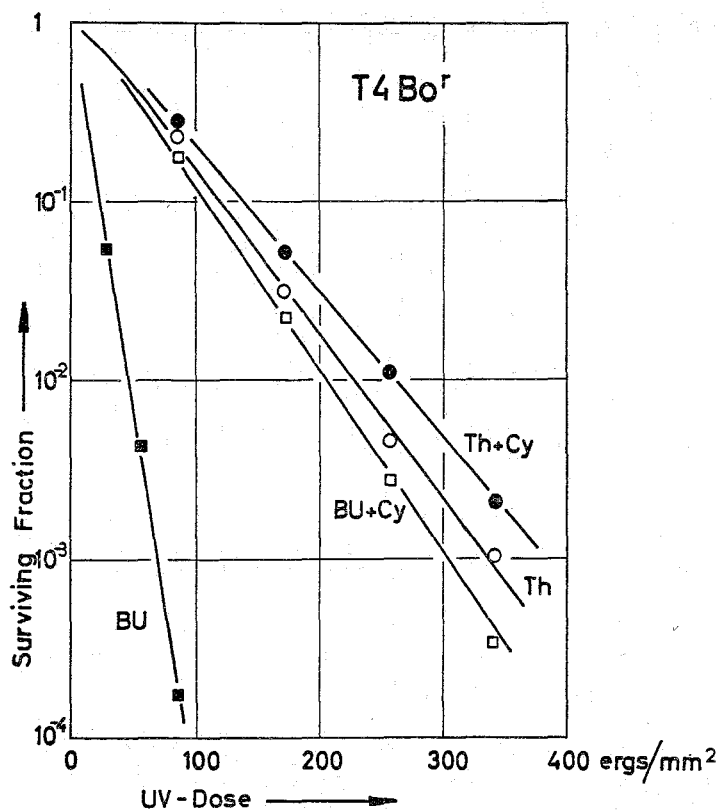

Fig. 1. Surviving fraction of buffersuspended normal T4 Bo^r (Th) and 5-bromouracil substituted bacteriophage (BU) after irradiation with uv-light (2537 Å). The influence of M/100 cysteamine on the survival of normal (Th + Cy) and BU-phage (BU + Cy) is shown in corresponding curves. (In curve "BU + Cy" the specific absorption by cysteamine has been subtracted)

(1) Incorporation of 5-bromouracil into the DNA sensitizes phage T4 Bo^r against uv-light by a factor of 4 (curve BU and Th).

(2) The presence of M/100 cysteamine during uv-irradiation of normal phage alters the slope of the dose survival curve by only 10% which can be attributed to the uv-shielding effect of the sulfur (curve Th + Cy). Phage BU-T4 Bo^r, however, is desensitized effectively and shows nearly the normal radiosensitivity of a thymine-containing phage (curve BU + Cy; corrected for the shielding effect), i.e. u-gene reactivation is fully restored.

2. Effect of UV-irradiation on DNA-deoxyribose

Fig. 2 shows the loss of deoxyribose in the DNA of BU-T4 Bo^r after uv-irradiation of the phage particle (curve BU). Destruction is expressed in percent of sugar of non-irradiated phage analysed under the same experimental conditions. The accuracy of the results is about ±2% in terms of %-deoxyribose destruction. For the normal phage (curve Th) and the BU-T4 Bo^r irradiated

in the presence of M/100 cysteamine (curve BU + Cy) the sugar degradation is by a factor of at least 4 lower than for the BU-phage irradiated without the protective compound present. After irradiation of BU-phage in the presence and absence of cysteamine we found uracil as the major photoproduct. In both cases, however, the amount of BU degraded or converted to uracil was the same within the limits of the experimental error.

To calculate the number of deoxyribose molecules changed per BU-phage lethal hit, we are using the following data: 11 ergs/mm^2, the dose required to
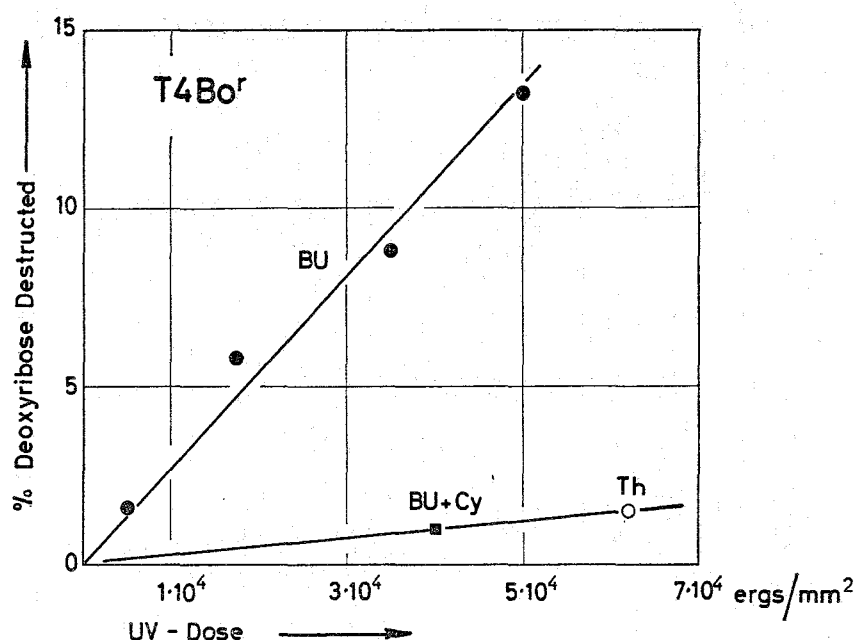

Fig. 2. Dose-effect-curve of the destruction of deoxyribose in the DNA after irradiation of phage T4 Bo^r particles with uv-light (2537 Å). The effect of irradiation on normal phage (Th) is compared with the damage occuring in BU-phage in the absence (BU) and presence (BU + Cy) of M/100 cysteamine

inactivate BU-T4 Bo^r to a survival of e^{-1}, i.e. the 37%-dose (D_{37}). Percentage of deoxyribose destroyed per D_{37} is 0.003% as obtained form the slope of the curve of Fig. 2. Assuming a DNA content per phage of $130 \cdot 10^6$ daltons (Rubinstein et al., 1961) 50% purine-bound deoxyribose amounts to $2.1 \cdot 10^5$ deoxyribose molecules. The number of pentose molecules harbouring a lesion due to photochemical reactions of BU is then 6.3 per phage lethal hit.

Discussion

Although most interesting photochemical results have been reported on the pure base iodouracil uv-irradiated in the presence of cysteamine and a scheme of reaction between both compounds was proposed (Rupp and Prusoff, 1965), studies in this direction on *whole* DNA were missing in the literature. As a starting point for the discussion of our results on BU-DNA we shall take the fact that uracil is the main photoproduct of BU, but is formed in equal amounts if the irradiation is carried out in the presence or in the absence of cysteamine. The same holds for the total amounts of BU destroyed. On the other hand in the presence of cysteamine the sensitizing effect of BU on the plaque-forming ability of phage is abolished. These results led us to consider more closely the details of the reaction transforming BU into U, i.e. where does the H-atom originate

which replaces the Br-atom in the pyrimidine ring. Possible sources for the H-atom would be the hydration water surrounding the DNA, the phage protein, the neighbouring bases and the deoxyribose moiety. As has been shown recently the uracil radical formed during uv-irradiation of an aqueous solution of BU does not attack water but recombines to form di-uracil (WANG, 1966). Hydrogen abstraction from the protein could be supposed to be a rather negligable damage to the phage. From the other two possible sources of H-atoms the bases seem to be much less likely than the pentose residues because of their known properties rather to add than to furnish H-atoms. Thus from the previously known results the hypothesis could be deduced, (1) that during uv-irradiation of BU-phage without protective compound present U-formation is accompanied by deoxyribose destruction, (2) that the protective action of cysteamine consists in furnishing the H-atom to the U-radical and thus preventing the destruction of deoxyribose. Inherent to this hypothesis is the assumption that U is a non-lethal base when it replaces BU or thymine which seems reasonable in view of the existence of a DNA from *Bac. subtilis* phage containing U in the place of thymine (TAKAHASHI and MARMUR, 1963).

Our results on the destruction of the pentose following uv-irradiation of BU-phage and protection by cysteamine lend support to the above hypothesis. Work is in progress to also elaborate a method for the determination of the pyrimidine-bound deoxyribose, in order to gain more quantitative information on the relation between total deoxyribose destroyed and U formed.

As regards the mechanism of the aforementioned reaction it is interesting to note that in uv-irradiated BU-DNA radicals have been observed which were attributed to the presence of BU (KOEHNLEIN and HUTCHINSON, 1966). From the observations discussed above a reaction scheme can be drawn for the BU sensitization and the influence of protective radical scavengers. Following uv-absorption BU is decomposed into the U-radical and a Br-atom. U is formed after H-abstraction from the pentose moiety, while supplementary damage could be caused by Br-atom. In the presence of protective compounds, however, the radicals interact with these compounds and are rendered harmless to the deoxyribose.

The fact that in phage T4 Bo^r containing BU u-gene reactivation is abolished and is restored when the phage is uv-irradiated in the presence of cysteamine now logically leads to the assumption that a necessary condition for this reactivation is an *undamaged* sugarphosphate backbone of the DNA. Although no experimental work on PHR and HCR has been done on the phage used in this study, it was shown, however, that in BU-T1 phage these types of reactivation are only working when the phage has been irradiated in the presence of a protective compound like cysteamine (HOTZ, 1963, 1964). We therefore tentatively conclude that in all these cases an undamaged backbone is necessary for the action of enzymes reactivating DNA lesions. An important question still open for discussion is: Why are the reactivating enzymes able to handle the photoproducts of BU with the same efficiency as the photoproducts of thymine if irradiation is performed in the presence of cysteamine? Three explanations can be given. (1) The quantum yield for the formation of BU-photoproducts is almost equal to the quantum yield of thymine photoproducts, or (2) the quantum yields are different

and the efficiency of reactivation is different, so that the same net result is observed, or (3) the known photoproducts of thymine, in principal the dimers, have little or nothing to do with the reactivable uv-damage. The first two conclusions are very unlikely. For the last mentioned one, however, evidence is accumulating from studies on the specific substrate for photoreactivating and host-cell reactivating enzymes which do not offer any positive support for the dimer-hypothesis, i.e. thymine dimers being the principal reactivable uv-radiation damage in DNA (Rupert, 1964; Sauerbier, 1964b; Harm, 1965).

In summary, experimental evidence for the destruction of DNA-deoxyribose in uv-irradiated BU-T4 Bo^r bacteriophage is given. The damage to the pentose as well as the sensitizing effect of the base analogue observed in dose-survival curves of phage can be eliminated by irradiation in the presence of M/100 cysteamine i.e. u-gene action is nearly normal. A working hypothesis is put forward to explain the blocking effect of BU on enzymes reactivating DNA-lesions as well as the mechanism of avoiding this block by cysteamine.

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