DOSE DEPENDENT DECREASE IN EXTRACTABILITY OF DNA FROM BACTERIA FOLLOWING IRRADIATION WITH ULTRAVIOLET LIGHT OR WITH VISIBLE LIGHT PLUS DYE*

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Received May 3, 1962

In 1928 Gates pointed out the probable relation between the bactericidal effectiveness of the various wave lengths of ultraviolet light (U.V.) and the absorption of U.V. by deoxyribonucleic acid (DNA). In 1941, Hollaender et al. described changes in the macromolecular properties of DNA due to U.V. irradiation. Sinsheimer and Hastings (1949) reported that uracil and cytidylic acid can undergo reversible hydration in U.V. Setlow and Doyle (1954) and Baranowska and Shugar (1960) have reported the U.V. crosslinking of dry films of DNA. In 1960 Beukers and Berends reported that U.V. can cause the dimerization of thymine and Wacker et al. (1960) found this to occur in vivo (presumably intrachain). Marmur and Grossman (1961) have reported the interchain crosslinking of DNA by U.V. The present report deals with a new phenomenon: a dose dependent decrease in the extractability of DNA when bacteria are irradiated with U.V. This response is analytically many times more sensitive than in vivo thymine dimer formation (Smith, 1962). This phenomenon also occurs when the bacteria are sensitized with acridine orange and exposed to visible light, but does not seem to be caused by x-irradiation.

METHODS

E. coli B or B/r were grown in sulfanilamide medium and E. coli B, T^- (W4516) was grown in mineral medium (Kaplan, Smith and Tomlin, 1962).

*This investigation was supported by Grant #C-2896 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

All cultures were supplemented with 1.2 mg of thymine-2-C-14 (25μc) per 100 ml of culture. Cultures of E. coli 15T⁻A⁻U⁻ were cultured as previously described (Maaloe and Hanawalt, 1961) except that thymine-2-C-14 (6.3μc) was substituted for unlabeled thymine. The cells were collected by centrifugation and suspended in 80 ml of 0.1M phosphate buffer (pH 6.8). 10 ml (10^{10} cells) were placed in each of 8 petri dishes (100 mm dia.) and irradiated for various times with shaking under an unfiltered General Electric germicidal lamp (G8T5) whose output was 1100 ergs/mm^2/min at 43 cm distance as measured by uranyl oxalate actinometry (Bowen, 1946). The cells were again collected by centrifugation and the DNA isolated by a modification of the method of Smith and Kaplan (1961). The cells were suspended in 14.5 ml of 2% sodium lauryl sulfate plus 0.5 ml 1 M sodium citrate (pH 7) and stirred slowly at room temperature for 60 minutes. The solution was precipitated with 2 volumes of cold 95% ethanol to remove small molecular weight thymine compounds. The mixture stood in ice for 20 minutes with frequent mixing and was then centrifuged for 30 minutes at 2000 RPM (International Centrifuge), decanted and drained. The precipitate was washed in cold 70% ethanol - 0.1 M NaCl and again centrifuged, decanted and drained. The precipitate was suspended in 10 ml of 2% sodium lauryl sulfate and stirred for 60 minutes at room temperature. The amount of DNA in this solution (measured by the amount of thymine-2-C-14 present) was determined by counting a 0.1 ml aliquot on a paper disc in a liquid scintillation counter (Mans and Novelli, 1960). A further aliquot was treated with an equal volume of 1 M KCl. The mixture was shaken occasionally over a period of 30 minutes and then centrifuged for 30 minutes at 2000 RPM (4°) and the supernatant filtered by gravity. The DNA and RNA appear in the supernatant while the detergent and protein appear in the precipitate. The amount of radioactivity in this supernatant compared to the amount in the detergent solution before treatment with KCl gives a measure of the recovery of free DNA. The recovery of DNA from unirradiated bacteria is quite constant from experiment to experiment

but differs from strain to strain. Thus, one recovers about 90% of the DNA from unirradiated E. coli B and B/r but only 82% from strain T^- (W4516).

RESULTS AND DISCUSSION

Figure 1 shows the effect of increasing doses of U.V. on the amount of DNA essentially free of protein that can be extracted from bacteria (unirradiated controls plotted as 100%). Thirty percent of the DNA is about 7 times more sensitive to U.V. than the remainder. The amount of the DNA that is lost from the supernatant due to irradiation can be quantitatively accounted for in the detergent-protein precipitate. At the 99% killing dose for E. coli B/r (1800 ergs/mm^2) only 0.1% of the thymine in the DNA is converted to the thymine dimer (Smith, 1962) yet the same dose renders 11% of the DNA unextractable. It would seem therefore that since this effect of U.V. is analytically many times more sensitive than thymine dimer formation it should have a proportionally greater biological significance. However, it does not seem to be related to bacterial radiation sensitivity and resistance because the responses of E. coli B and B/r are identical. Using E. coli B, T^- we have found that conditions which photoreactivate colony formation (20 fold increase in survivors) do not reduce the U.V. lesion measured here. The DNA in penicillin protoplasts (Lederberg, 1956) of E. coli B, T^- showed the same response to U.V. as had been noted for DNA in intact cells (Figure 1). This indicates that the decreasing recovery of DNA is not due to a failure of the cells to lyse after irradiation. In one experiment, the DNA of cells (T^-) in log phase growth appeared about 6% more sensitive than that of stationary phase cells (24 hour culture). After a dose of 2.9×10^4 ergs/mm^2 the thymine dimer content of T^- was the same for the isolatable DNA as for the DNA that precipitated with the protein (1.9% of incorporated thymine-2-C-14 or -methyl-C-14). A second photoproduct arising from thymine, which was

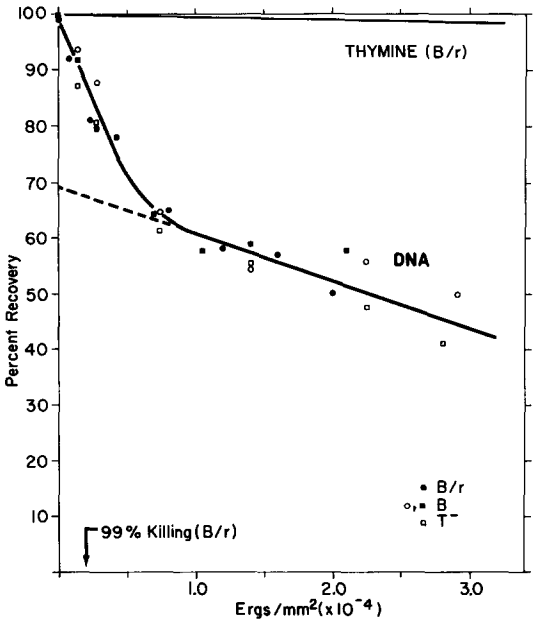

Figure I. The Extractability of DNA From E. coli Following Irradiation with Increasing Doses of Ultraviolet Light.

Thymine-2-C-14 labeled E. coli were grown to saturation (24 hours) and then irradiated with increasing doses of U.V. and extracted with sodium lauryl sulfate to isolate the DNA. The graph represents the recovery of free DNA. The DNA not isolated remained associated with the denatured proteins. For comparison, data of Smith (1962) are included on the rate of formation of thymine dimer in E. coli B/r with increasing dose of U.V. (plotted here as the loss of recovery of thymine).

present in higher concentration in the DNA precipitated with the protein, is currently under investigation.

Since the triple mutant of E. coli ($15T^{-}A^{-}U^{-}$) requires thymine, arginine and uracil, one can selectively inhibit DNA, RNA or protein synthesis (Kanazir et al., 1959). When cells were grown for 110 minutes in the absence of arginine and uracil (Maaloe and Hanawalt, 1961) these cells showed a response to U.V. which fell on the line described in Figure 1. However, if they were first grown on radioactive thymine, then on a thymine-less medium for 60 minutes, and then irradiated with various doses of U.V., it was found that 60% of the DNA in these cells was of the more sensitive type (against 30% under the usual conditions) and the lower portion of the

curve was essentially parallel to the normal curve. In a second experiment, samples of cells were taken after the bacteria had been grown for various time intervals in the absence of thymine and after restoration of thymine for various time intervals. All the samples were irradiated with an equal dose of U.V. (1.44×10^4 ergs/mm^2). The amount of DNA that was isolatable decreased with increasing intervals of incubation in the absence of thymine, and slowly returned to the normal response with increasing times of growth in the presence of thymine.

After an X-ray dose of 1Kr there was about a 5% loss in extractable DNA (E. coli B, T^-) but doses up to 40Kr did not alter this figure. When acridine orange was introduced at a dilution of 1 in 7×10^5 to a cell population of 2×10^9 per ml (E. coli B, T^-) and this population irradiated for periods up to an hour with a 500 W flood lamp (Freifelder and Uretz, 1960), it was found that after 15 minutes the surviving fraction was reduced to 7% and the amount of extractable DNA dropped to 70%. Progressively more DNA was rendered unextractable after 30, 45 and 60 minutes of visible light irradiation; after 60 minutes only 20% of the DNA was extractable. On the basis of dose to produce the same reduction in colony formation, visible light plus dye appears to render a larger percentage of the DNA unextractable than does U.V.

For U.V.-damaged DNA to be non-extractable in the above isolation procedure, it must either combine with protein and then be carried down when the denatured proteins are precipitated, or must combine with the detergent and be carried down when the detergent is precipitated. The following evidence supports the concept of a U.V.-induced interaction between the DNA and protein*. When the DNA-protein-detergent precipitate from a U.V. irradiated culture was freed of detergent and then homogenized in 55% CsCl and banded in the ultracentrifuge, more than 80% of the DNA remained associated with the protein (floated on top). Treatment of this

*Also observed by P. Alexander and H. Moroson (personal communication).

material with trypsin, however, yielded free DNA. Bacteria ($15\bar{T}\,\bar{A}\,\bar{U}$) containing twice their complement of protein (and RNA) but only a single complement of DNA were twice as sensitive to loss of extractable DNA in this system. DNA irradiated *in vitro* (8.6×10^4 ergs/mm^2) does not show a loss when subsequently submitted to the isolation procedure. However, when DNA was irradiated in the presence of bovine serum albumin, a dose dependent loss of DNA was observed. The interaction of the DNA and protein *in vivo* following U.V. does not appear to be due to a covalent link: when irradiated cells were ground with alumina, extracted with saline and then either banded in CsCl or treated with detergent, no indication of crosslinking of DNA and protein was detected. It would appear therefore that the detergent must stabilize the U.V. lesion only if it is also used as the lysing agent.

Although it is still to be determined whether the detergent stabilizes the U.V. lesion by direct chemical interaction or whether it prevents recovery from the lesion (allowed by salt extraction), the fact remains that the DNA in a bacterial culture can be divided into two populations, one of which is about seven times more sensitive to U.V. than the other. This biphasic response need not imply two chemically different kinds of DNA, but could equally well reflect differences in the physical form of the DNA or in its protein milieu. Since this response to U.V. is analytically many times more sensitive than thymine dimer formation we suggest that it must play a significant role in the U.V. inactivation of bacteria.

ACKNOWLEDGMENT

I would like to acknowledge the valuable technical assistance of Mrs. Mary E. O'Leary, and to thank Dr. Philip C. Hanawalt for supplying the cultures of E. coli $15\bar{T}\,\bar{A}\,\bar{U}$ and Dr. Robert B. Uretz for helping us set up the dye plus visible light experiment.

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