Fate of Thymine-containing Dimers in the Deoxyribonucleic Acid of Ultraviolet-irradiated Bacillus subtilis

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The fate of ultraviolet-induced, thymine-containing dimers in the deoxyribonucleic acid (DNA) of Bacillus subtilis was investigated in both the wild type (UVB) and an ultraviolet light-sensitive (UV8) mutant. During incubation in the dark, dimers were excised from the DNA of the UVB- B. subtilis, but remained in the DNA of the UV8 mutant. About 40% of the excised dimers recovered in the wild type were in the acid-soluble fraction; the remainder were in the incubation medium. A UV8 mutant of Escherichia coli K-12, shown previously to be defective in dimer excision, was irradiated with ultraviolet light and incubated under visible light for 3 hr. About 65% of thymine-containing photoproducts were removed from the DNA. These photoproducts were not recovered in the acid-soluble fraction. In comparison, the UV8 mutant of B. subtilis lost only 13% of such photoproducts from DNA when exposed to light under the same conditions.

Irradiation of bacteria with ultraviolet light (UV) produces a variety of pyrimidine dimers in their deoxyribonucleic acid (DNA; 15). In Escherichia coli, there are two distinct mechanisms for repairing this damage; one operates in the presence of light (4), and one functions in the dark (20). The photoreactivating repair system is enzymatic (4, 10), requires light of wavelength 3,200 to 4,000 A (8), and functions by splitting the pyrimidine dimers in situ (16, 21). It has been reported that Bacillus subtilis fails to undergo photoreactivation as measured by an increase in survival of UV-irradiated cells after exposure to visible light (A. Kelner, Radiation Res. Soc. Abstr., 1965, p. 35). Dark repair involves the excision of the intact dimers and is accompanied by degradation of the bacterial DNA (1, 2, 7, 14, 18).

A number of UV-sensitive mutants of E. coli have been isolated and found to be defective in their ability to excise dimers during incubation in the dark (2, 5, 7, 14). They were also found to be incapable of repairing UV-irradiated T1 phage (6, 10, 12).

Recently, a UV-sensitive mutant of B. subtilis was isolated which is also defective in repairing UV-irradiated phage (13). It was the purpose of these experiments to determine the fate of UV-induced thymine-containing photoproducts in the DNA of both the normal and UV8 mutant of B. subtilis during post-UV incubation in the dark or under visible light. (UVB and UV8 will be used to denote UV-sensitive and UV-resistant phenotypes; uvr will be used as the symbol for a genetic locus controlling pyrimidine dimer excision and UV sensitivity.) Experiments showed that thymine-containing dimers were excised in the dark from DNA of the normal strain but not from the UV8 mutant. Incubation in visible light had no significant effect on the number of dimers remaining in the DNA of the UV8 B. subtilis mutant. In contrast, a UVB mutant of E. coli lost 65% of thymine-containing dimers from DNA when incubated in visible light after UV irradiation.

MATERIALS AND METHODS

Bacterial strains. A transformable strain of B. subtilis, JB1-49 (ind- his+ cys- uvr+), and a UV-sensitive mutant, JB1-49 23 (ind+ his+ cys+ uvr-), were kindly supplied by Bernard Strauss. The UV8 mutant was obtained by transformation of JB1-49, by use of DNA carrying a uvr- mutation as a donor (13). A growth requirement for low concentrations of thymine was introduced into both strains by the methods of Stacey and Simson (17). A thymine-requiring UV8
fluoracetic acid
soluble

The minimal growth medium contained (per liter): (NH₄)₂SO₄, 2 g; sodium citrate, 1 g; MgSO₄, 0.2 g; glucose, 5 g; KH₂PO₄, 15 g; and KH₂PO₄, 6 g. The pH was adjusted to 7.2 with KOH. Cells were grown to late log phase in 6.0 ml of minimal medium supplemented with 0.6 ml of 5% Casamino Acids, 0.12 ml of 5% tryptophan, and 0.2 ml of methyl-H-thymine (17 μg; specific activity, 14.5 c/mmole; New England Nuclear Corp., Boston, Mass.). The culture had a generation time of 50 min.

Ten minutes before termination of growth, 100 μg of nonradioactive thymidine was added. The cells were washed twice in minimal medium by centrifugation and were then suspended in minimal medium for irradiation. More than 90% of the radioactivity of thymine-containing dimers to thymine was observed between the two samples, indicating that no selective loss of either dimers or thymine occurred upon adsorption and elution from charcoal.

RESULTS

Table 1 shows the numerical results of these experiments. Upon incubation, there was a decrease in photoproducts relative to thymine only in the UV© JBI-49 strain. In the UV© strain, JBI-49 23, no decrease in photoproducts relative to thymine was observed during incubation in the dark. Thus, as in E. coli, the UV© strain of B. subtilis is able to remove preferentially photoproducts from the DNA during incubation in the dark, whereas the UV© strain is incapable of doing so.

If the disappearance of photoproducts from the DNA occurred by excision as in E. coli, then the pyrimidine dimers should appear in the acid-soluble fraction after incubation. However, all of the radioactivity initially present in pyrimidine dimers could not be accounted for in the acid-soluble and -insoluble fractions, and it was found that approximately 60% of the excised photoproducts recovered after incubation appeared in the medium.

To test whether dimers still attached to phosphate were passing out of the cell, the incubation medium was adsorbed to and eluted from charcoal. Concentrated samples were chromatographed after elution in butanol-acetic acid-water without trifluoroacetic acid hydrolysis. Under these conditions, phosphorylated compounds remain close to the origin (2). It was found that over 90% of the radioactivity appeared as free thymidine or thymine, which are poorly separated in this system; the remaining activity was clustered in the region of the origin. The origin region was eluted, hydrolyzed, and chromatographed. Three radioactive peaks were observed: two corresponded to the Rf values for the thymine-containing photoproducts, and the third corresponded to the value for thymine (2). The photoproducts were eluted, UV-irradiated in aqueous solution at a dose of 10⁴ erg/mm², and chromatographed. After this treatment, the radioactivity migrated at the same Rf as did thymine. These results indicate that the excision products containing the dimers remain phosphorylated in their passage through the cell into the medium.

The numerical results of the photoreactivation experiment can be seen in Table 2. Incubation in the dark had relatively little effect on the dimer-to-thymine ratios in acid-insoluble DNA obtained from the sensitive strains of either B. subtilis or E. coli K-12. However, exposure to...
The results of these experiments indicate that the dark-repair mechanism in *B. subtilis* exhibits properties similar to *E. coli* with regard to excision of UV-induced photoproducts. There is a preferential removal of thymine-containing dimers from the DNA upon incubation of the UV\(^ \text{R} \) strain in the absence of light, whereas the UV\(^ \text{R} \) strain is deficient in the excision mechanism.

**DISCUSSION**

visible light reduced the ratio in *E. coli* K-12 by 65% and in *B. subtilis* by 13%.
The loss of photoproducts from DNA in strain J81:49 (uvr+) during incubation is recorded in Table 1. About 75% of the photoproducts were released from DNA during incubation, but only 35% of the thymine radioactivity was solubilized during the same period. The photoproducts could be released by degradation of 75% of the DNA followed by 40% reincorporation of breakdown products (but not photoproducts). However, it seems unlikely that appreciable incorporation could occur after degradation of 75% of the DNA; it is much more likely that the photoproducts are excised, as suggested in E. coli and Micrococcus radiodurans.

In addition, direct evidence for dimer excision has recently been obtained with a partially purified cell-free extract prepared from Micrococcus lysodeikticus (W. L. Carrier, and R. B. Setlow, Biochim. Biophys. Acta, in press).

One difference between E. coli and B. subtilis is the presence of phosphorylated photoproducts in the postirradiation incubation medium of B. subtilis. A similar finding has recently been reported for M. radiodurans (1).

Whereas in E. coli there is a decrease of almost 65% of thymine-containing dimers from DNA during exposure to light for 3 hr, the results in Table 2 show that, in B. subtilis, the amount of dimers in DNA fell by only 13%, which is probably within the limits of experimental error. Thus, at this dose of UV irradiation, B. subtilis shows no significant removal of dimers by photoreactivation. There was a decrease in the dimer-to-thymine ratio of 20% in the UV8 E. coli K-12 AB2500 strain upon incubation in the dark. This may be indicative of some slight excision occurring in this strain at the UV dose administered to the cells. The decrease in the ratio of dimer to thymine is significantly less than that observed for the UV8 strain of E. coli K-12 incubated after irradiation under the same conditions (2).

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LITERATURE CITED


