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Postincision Steps of Photoproduct Removal in a Mutant of *Bacillus cereus* 569 That Produces UV-Sensitive Spores

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An excision-defective mutant of *Bacillus cereus* 569 is normal in incision and repair synthesis, but rejoining of incision breaks is defective, resulting in accumulation of low-molecular-weight DNA after UV irradiation. The defect in removal of photoproducts by exonuclease after incision renders both vegetative cells and dormant spores of the mutant sensitive to UV. A similarity is indicated to the *uvrD* mutation described recently in *Escherichia coli*.

Basic knowledge of DNA repair processes has come primarily from experiments with bacterial cells exposed to UV radiation and from biochemical studies on DNA replication and repair (3, 4, 8, 10–12). In vegetative cells, the mechanisms dealing with UV lesions are dark repair processes: excision repair and post-replication repair (20). In spores, the spore-specific photoproduct is eliminated predominantly in situ and partially by excision repair (13, 16).

Three groups of mutants producing UV-sensitive spores have been described in *Bacillus subtilis*: the *rec* mutants, the *pol* mutants, and the double mutants defective in both excision repair (*uvr*) and in in situ destruction of the spore-specific photoproduct (*ssp-1*) (12, 13, 15–17).

Recently, we isolated, by single step mutagenesis, a mutant of *Bacillus cereus* 569 sensitive to UV in both vegetative and sporal stages (22). The UV-sensitive mutant designated *B. cereus* 2422 is capable of in situ removal of the spore-specific photoproduct but is deficient in excision of both cyclobutane thymine dimers and spore-specific dimers.

The purpose of this investigation, therefore, was to examine the incision and postincision steps of pyrimidine dimer removal in this UV-sensitive mutant. The bacterial strains and media used throughout this study were described previously (22).

Cells able to excise UV damage undergo post-irradiation degradation of the DNA, manifested as a release of nucleotides into the acid-soluble fraction of the cell and into the surrounding medium (2). A strain that is incision defective (*uvrA, B, C*) would be expected to show decreased degradation after UV irradiation and incubation (17, 22). A mutant that is defective in a step after incision is expected to show variable levels of increased degradation (23). In our experiments, DNA degradation was estimated by measuring [^{3}H]thymidine radioactivity in the acid-soluble fraction. The total radioactivity in the cells was 250,000 cpm for vegetative cells and 100,000 cpm for dormant spores. Degraded DNA was expressed as the percentage of the radioactivity in a 1-ml control sample hydrolyzed in hot 10% trichloroacetic acid (90°C for 30 min). Both the wild type and the mutant undergo DNA degradation after exposure to UV irradiation (Fig. 1). The mutant 2422 (Fig. 1B and D) showed increased DNA degradation in comparison with the wild type (Fig. 1A and C).

Both post-irradiation DNA degradation and repair synthesis cannot proceed without incision. The UV-induced incision of DNA was tested by using the procedure described by Hadden (5). Cells in exponential phase labeled with [^{3}H]thymidine were UV irradiated with 9 J/m^2 and were incubated in complete medium with and without 6-(*p*-hydroxyphenylazo)-uracil (HPUra, a specific inhibitor of semi-conservative DNA synthesis [1]). The treated cells were lysed and layered on a 5-ml linear alkaline sucrose gradient. A significant amount of incision occurred in both the mutant (strain 2422) and the wild type (strain 569). The addition of HPUra to the incubation medium had no effect on the rate of incision.

The reinitiation of DNA synthesis after UV irradiation depends on the dark repair. UV-

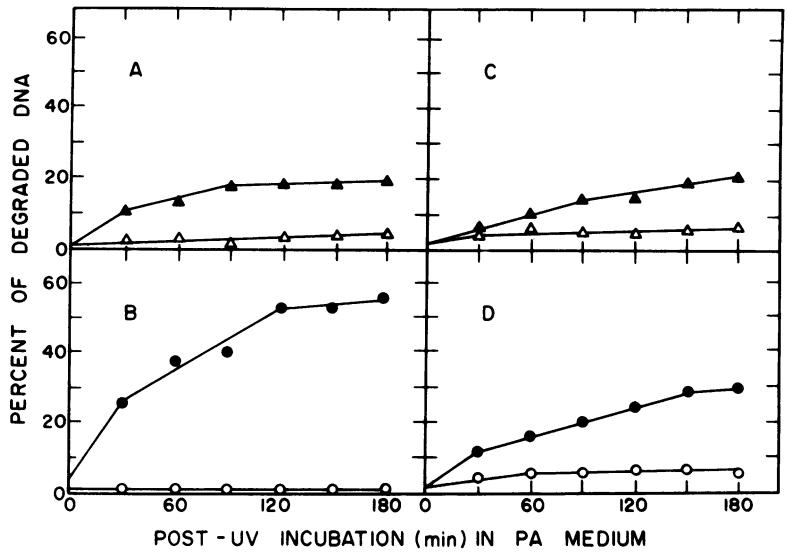

FIG. 1. DNA degradation of vegetative cells (A and B) and of dormant spores (C and D) after exposure to UV irradiation (20 J/m^2 for vegetative cells and 200 J/m^2 for dormant spores). Symbols: △,○ unirradiated; ▲,●, UV irradiated; (A and C) *B. cereus* 569 (▲,△); (B and D) *B. Cereus* 2422 (●,○).

induced pyrimidine dimers may act as blocks of DNA synthesis, and the excision of dimers is a necessary step in the reinitiation of DNA synthesis (18). The enzyme DNA polymerase I binds preferentially to nicks and performs both excision of the damaged portion and repair replication (4). To measure repair synthesis directly, we followed the incorporation of [^{3}H]bromouracil into the light parental DNA labeled with [^{14}C]thymine, using the method described by Hanawalt et al. (6, 7). Exponentially growing cultures of *B. cereus* 569 and 2422 labeled with [^{14}C]thymine were irradiated with 8 J of UV per m^2, and repair was allowed to proceed in the presence of [^{3}H]bromouracil. Analysis of the isolated DNA in cesium chloride equilibrium density gradients provides a measure of the amount of density label incorporated, as well as its distribution among the DNA fragments. To isolate the unreplicated, repaired DNA from newly replicated heavy-density and hybrid-density DNA, the peak fractions of ^{14}C-labeled light-density DNA were rebanded by centrifugation in neutral cesium chloride (6). UV irradiation stimulated incorporation of [^{3}H]bromouracil, repair label, into the light-density DNA of both the mutant (strain 2422) and of the wild type (strain 569) (Fig. 2). From the results described above, we conclude that the UV-sensitive mutant (strain 2422) is capable of post-irradiation repair synthesis by DNA polymerase I.

Rejoining of DNA breakage after UV irradiation was examined by using the procedure described by Hadden (5). Growing cells of *B. cereus* 569 were irradiated and returned to growth medium. The DNA was rapidly nicked, as shown by the shift of the DNA profile (Fig. 3A). After 30 min of incubation, a shift towards higher molecular weight was observed (Fig. 3B). After 60 min (Fig. 3C), the DNA profile was similar to that of the unirradiated control. Under the same conditions, the irradiated mutant (strain 2422) exhibited a shift to the lower-molecular-weight DNA (Fig. 3D), and the sedimentation profile remained unchanged, even after 60 min (Fig. 3F). Thus, the mutant (strain 2422) exhibits very little rejoining of the single-strand breaks.

The *uvr* mutants previously described in *B. subtilis* are deficient in incision steps (12, 14), and our present results indicate the *B. cereus* 2422 is defective in a postincision step of the photoproduct removal, which plays a major role in the survival of UV-irradiated spores. Since the *ssp* mutation was not isolated in *B. cereus* 569, the contribution of the in situ removal of spore photoproduct cannot be determined.

From our previous and present findings, that the UV-sensitive mutant is (i) resistant to alkylating agents (22) and (ii) capable of incision at the damaged site, we exclude the possibility of deficiency in either DNA-glycosylase or AP-endonuclease. The deficiency seems to be in a step after the incision step. Since the mutant (strain 2422) performs repair synthesis at nicks on damaged DNA but fails in rejoining the incision breaks, we suggest the existence of free 3′-OH and a 5′-phosphorylated thymine:thymi-

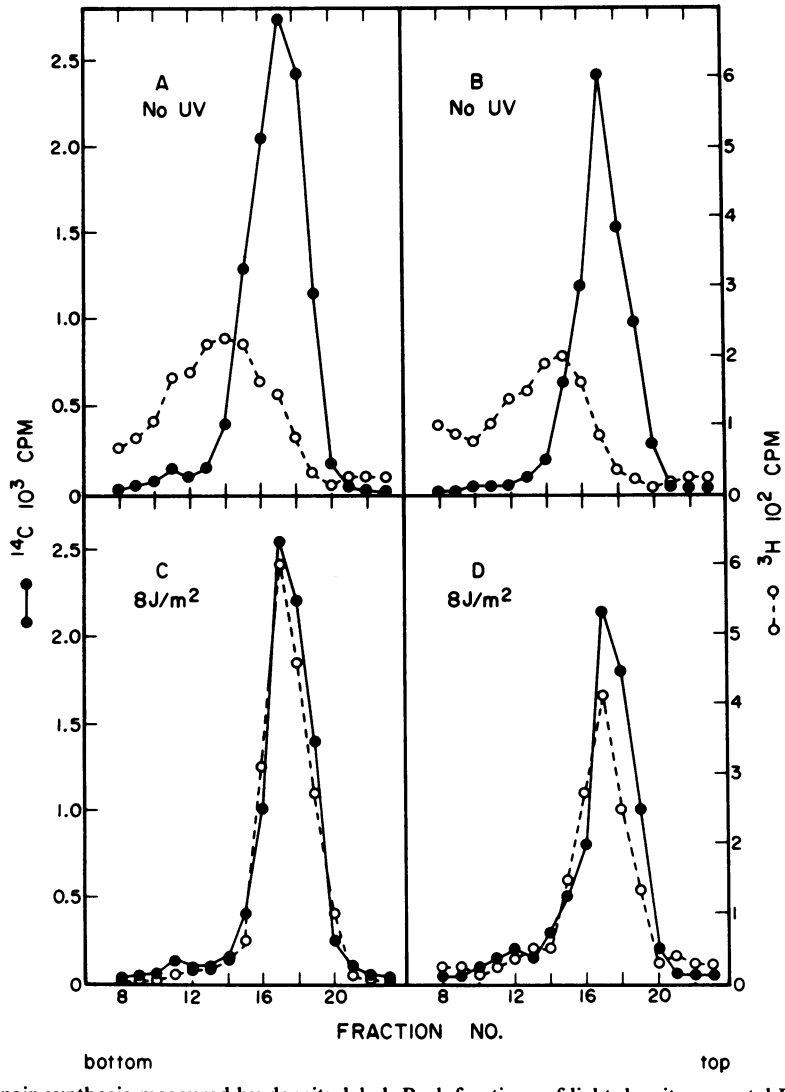

FIG. 2. Repair synthesis measured by density label. Peak fractions of light-density parental DNA, from the neutral CsCl gradients, were pooled and rebanded by centrifugation in neutral CsCl. Profiles of radioactivity recovered from these (rebanded) gradients are shown. (A) *B. cereus* 569 (wild type), unirradiated; (B) *B. cereus* 2422, unirradiated; (C) *B. cereus* 569, 8 J/m^2; (D) *B. cereus* 2422, 8 J/m^2. Symbols: ●, ^{14}C prelabel; ○, ^{3}H repair label.

dylate dimer at the incision site (4). Whereas the 3′-OH serves as primer for action of DNA polymerase I (Fig. 3), the 5′-phosphorylated thymine:thymidylate dimer must be removed by a 5′ → 3′ exonuclease. Thus, the failure to join the incision breaks by ligase might be due to the absence of exonucleolytic activity of DNA polymerase I or III or of exonuclease VII, which have been implicated in the excision process.

Tang and Patrick (21) showed that DNA polymerase I is essential for liquid holding recovery. The absence of liquid holding recovery in our mutant (22) indicates that the mutation might be in 5′ → 3′ exonucleolytic activity of DNA polymerase I.

An alternative explanation, described recently by Kuemmerle and Masker (9) in *Escherichia coli*, might be the absence of a protein which mediates conformational changes in DNA at the incision site. It is coded by the *uvrD* gene and is essential for completing the incision of photoproducts. The excision repair deficiency found by us in *B. cereus* 2422 may be similar to that described as *uvrD* in *E. coli*.

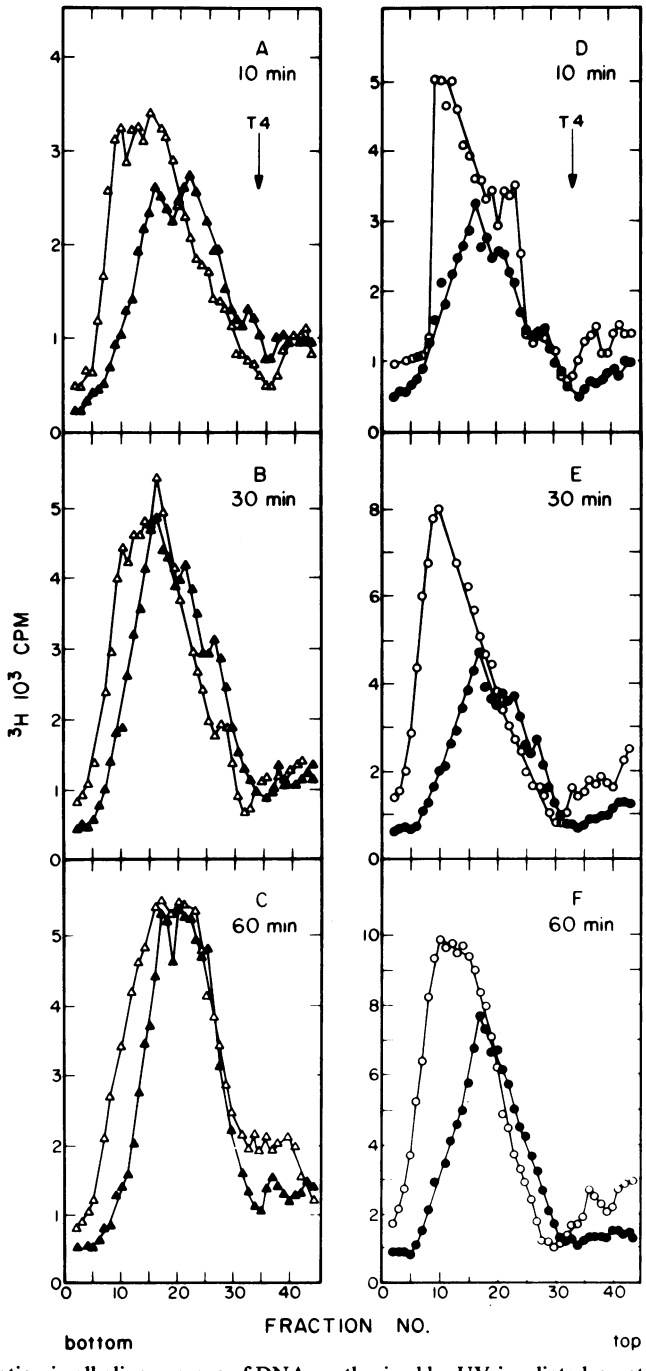

FIG. 3. Sedimentation in alkaline sucrose of DNA synthesized by UV-irradiated vegetative cells of *B. cereus* strains 569 (A, B, C) and 2422 (D, E, F). Exponentially growing cells labeled with [^{3}H]thymidine were washed, irradiated with 8 J of UV light per m^2, and incubated in complete medium supplemented with [^{3}H]thymidine. Samples were removed at appropriate intervals. Spheroplasts were prepared, and 100-μl portions were layered onto alkaline sucrose gradients. Symbols: △,○, unirradiated controls; ▲,●, UV irradiated.

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