UV Light Induction of Proteins in *Bacteroides fragilis* Under Anaerobic Conditions

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Far-UV irradiation of *Bacteroides fragilis* cells under anaerobic conditions resulted in the induction of a new 95,000-molecular-weight protein and the increased synthesis of two proteins with molecular weights of 90,000 and 70,000. The latter two proteins were synthesized in small amounts in unirradiated cells. The induction of a 37,000- to 40,000-molecular-weight protein was not observed in irradiated *B. fragilis* cells. Caffeine, which affected the survival of irradiated *B. fragilis* cells and reduced host cell-mediated UV reactivation, specifically inhibited the induction of the 95,000-, 90,000-, and 70,000-molecular-weight proteins. Sodium arsenite did not affect the induction of the three inducible proteins or the survival of irradiated *B. fragilis* cells.

The survival of *Bacteroides fragilis* cells after far-UV radiation (254 nm) is interesting as this organism is more sensitive to UV radiation in the presence of oxygen (9, 10, 28). In other bacteria, it has been shown that inactivation by far-UV light is independent of the presence of oxygen (31, 32). Our recent studies on the repair of irradiated phase indicated that under anaerobic conditions *B. fragilis* has an inducible host cell-mediated UV reactivation system (J. R. Parker, D. T. Jones, and D. R. Woods, submitted for publication). UV reactivation (Weigle reactivation) in *Escherichia coli* is entirely dependent on host cell functions (2). UV light damage in *E. coli* induces increased transcription at several specific loci and de novo protein synthesis which is required for the SOS responses (11, 13, 33). One of the prominent proteins induced by far-UV light and involved in SOS responses is the *recA* protein, which has a molecular weight between 37,000 and 40,000 (3, 6, 20, 25). The *recA* and *lexA* gene products regulate the expression of a set of damage-inducible (*din*) genes (11) whose products are required for some of the SOS responses. The *din* genes include the *uvrA* (11, 12), *uvrB* (4, 12), *sfiA* (8), and *umuC* (1) genes. Neither the existence of a similar *recA*-type protein nor the number and molecular weights of proteins induced by far-UV light in obligate anaerobic bacteria have been reported. We investigated the induction of proteins by far-UV light in *B. fragilis* under anaerobic conditions. The effects of caffeine and sodium arsenite on the induction of proteins by UV light were also investigated. In *E. coli*, caffeine has been reported to inhibit excision repair processes (5, 22, 23, 27, 29), but not the inducible *recA*-dependent repair system. Sodium arsenite is thought to inhibit *recA*-dependent steps in DNA repair in *E. coli* (21).

**MATERIALS AND METHODS**

*Bacteria and media.* These studies were carried out on a *B. fragilis* strain (BF-2) which has been described previously (17, 18) and was used in our previous studies on far-UV irradiation (9, 10, 28; Parker et al., submitted for publication). Brain heart infusion broth and agar, supplemented with hemin, menadione, and cysteine (7), were used for bacterial propagation at 37°C. Prerduced one-quarter-strength Ringer solution was used as a dilution buffer (10). Irradiation and radioactive labeling of the cells were carried out in a defined minimal medium (30) under stringent anaerobic conditions in an anaerobic glove box (Forma Scientific, Marietta, Ohio).

*UV irradiation and post-irradiation treatment.* Overnight cultures of *B. fragilis* cells in brain heart infusion broth were diluted 10-fold in minimal medium and reincubated until the culture reached a turbidity of 0.2 at 600 nm (1 × 10⁸ to 2 × 10⁸ colony-forming units/ml). Samples (9 ml) of the cultures were irradiated in open glass petri dishes with a Fluotest Piccolo Hanau Quartz germicidal lamp which emitted the majority of its output at 254 nm. The dose rate was measured with a Blak-Ray UV meter (model J-255; UV Products Inc., San Gabriel, Calif.), and samples were irradiated at a fluence rate of 1.0 J m⁻² s⁻¹. Survival curves of cells irradiated with increasing fluences were determined, and labeling experiments were routinely carried out with cultures irradiated to a survival level of 0.1%.

The cells (2 ml) were pulse-labeled for 10 min at 37°C by the addition of [³⁵S]methionine (Radiochemical Centre, Amersham, England) (10 μg/ml; 40 μCi/ml) at different time intervals (0, 15, 25, and 35 min) after UV irradiation. The cells were collected by
centrifugation with a microfuge, washed twice with the Ringer solution, and suspended at a 20-fold concentration of the original cell volume in electrophoresis buffer containing sodium dodecyl sulfate (SDS). The samples were then boiled for 2 min and either resolved immediately by polyacrylamide slab gel electrophoresis (PAGE) or stored at −20°C until required.

**Effect of caffeine and sodium arsenite.** The effect of caffeine and sodium arsenite on UV survival and the production of UV-induced proteins were determined. The minimal inhibitory concentrations for caffeine and sodium arsenite were 2.5 mg/ml and 130 μg/ml, respectively. Caffeine (1 mg/ml) or sodium arsenite (100 μg/ml) was added to the cultures immediately after irradiation.

**Electrophoresis and autoradiography.** The labeled proteins were subjected to discontinuous SDS-8.4% PAGE according to the methods described by Laemmli (14) and O’Farrell (19). The samples (20 μl) were stacked at 100 V and then resolved on 0.5 by 140 by 170-mm slab gels at 80 V for 7 h at 20°C. The gels were stained with Coomassie brilliant blue (0.05%, wt/vol), destained, washed, and dried. Labeled protein bands were visualized by exposing the dried gels to Kodak X-Omat MA X-ray film at −20°C for 28 days (15). Molecular weight markers were supplied by BDH Biochemicals Ltd. (Poole, England) and consisted of cross-linked polymers of a purified degradation product of myoglobin with a molecular weight range from 14,300 to 71,500. Purified human transferrin (molecular weight 90,000; Sigma Chemical Co., St. Louis, Mo.) was used as an additional marker. The autoradiograph strips were scanned with a Beckman DU-8 spectrophotometer with gel scanner attachment.

**RESULTS**

**UV induction of proteins.** *B. fragilis* cells irradiated in minimal medium at a fluence of 75 J m⁻² showed a level of survival of 0.1%. Under these conditions, filament formation or clumping did not occur and did not cause a sampling problem in the labeling experiments. The incorporation of [³⁵S]methionine into irradiated cells (0.1% survival) was linear over 10 min.

The induction of a new protein of molecular weight 95,000 (protein 1) and the induced synthesis of two other proteins with molecular weights of 90,000 and 70,000 (proteins 2 and 3, respectively) were observed after UV irradiation of *B. fragilis* cells under anaerobic conditions in eight independent experiments (Fig. 1 and 2). The 90,000- and 70,000-molecular-weight proteins were synthesized in small amounts in unirradiated cells. The production of these proteins increased over a 35-min period after UV irradiation (Fig. 1). Densitometric comparison of [³⁵S]methionine-labeled protein bands after SDS-PAGE of extracts from irradiated and unirradiated cells indicated that except for proteins 1, 2, and 3, the proteins showed relatively minor variations after UV irradiation (Fig. 2). The induction of a protein with a molecular weight between 37,000 and 40,000 was not observed in any of the eight different UV irradiation and autoradiograph experiments.

**Effect of caffeine and sodium arsenite.** We previously reported that caffeine reduced UV reactivation (Parker et al., submitted for publi-
cation) and caused a marked decrease in the number of surviving colony-forming units after UV irradiation under anaerobic conditions (9). The most pronounced effect of caffeine involved the reduction in the size of the shoulder of anaerobic UV survival curves. The addition of sublethal concentrations of caffeine immediately after UV irradiation specifically inhibited the induction of the 95,000-, 90,000-, and 70,000-molecular-weight proteins (Fig. 3). After treatment with caffeine, the 95,000-molecular-weight protein was absent, and the concentrations of the 90,000- and 70,000-molecular weight proteins were similar to those in unirradiated cells.

When cells were plated onto sodium arsenite plates (100 μg/ml) after irradiation under anaerobic conditions, no decrease in the number of surviving colony-forming units occurred (Fig. 4). Furthermore, there was no difference in the survival curves of B. fragilis cells irradiated aerobically and held under anaerobic conditions in a minimal salts solution (10) in the presence or absence of sodium arsenite (Fig. 4). The decreased survival of the cells irradiated aerobically but held anaerobically was due to the effect of air, which has been described previously (9, 10, 28). The addition of 100 μg of sodium arsenite per ml immediately after UV irradiation did not affect the production of the three UV-induced proteins (Fig. 3).

**DISCUSSION**

Exposure of B. fragilis cells to far-UV light resulted in the induction of one novel protein (molecular weight 95,000) and the increased synthesis of two proteins (molecular weights of 90,000 and 70,000) which were synthesized in small amounts in unirradiated cells. The anaerobe B. fragilis differs from E. coli in that a protein of molecular weight between 37,000 and 40,000, corresponding to the E. coli recA protein, is not observed after induction by far-UV light. Other proteins in E. coli which are induced by UV light include the uvrA, uvrB, and lexA proteins (molecular weights of 114,000, 84,000 and 25,000, respectively) (16, 24, 26). The roles of the three UV-induced proteins in B. fragilis are not known. We are attempting to isolate UV-sensitive and -resistant mutants in order to identify the functions of the three proteins.

Other important differences in the UV repair
processes of \textit{E. coli} and \textit{B. fragilis} involve the effects of caffeine and sodium arsenite. In \textit{E. coli}, excision repair and host cell reactivation are inhibited by caffeine, whereas \textit{recA}-dependent repair is thought to be inhibited by sodium arsenite (5, 21–23, 27, 29). UV survival and the induction of the three UV-inducible bands are not affected by sodium arsenite in \textit{B. fragilis}, whereas caffeine inhibits cell survival (9). UV reactivation (Parker et al., submitted for publication), and the induction of the three UV-inducible proteins.

The effect of oxygen on far-UV survival and the differences reported in this study suggest that the far-UV-induced repair systems in the obligate anaerobe \textit{B. fragilis} differ from those in \textit{E. coli}, which tend to be accepted as typical for bacteria in general.

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LITERATURE CITED