DNA Repair Systems in the Phototrophic Bacterium

*Rhodobacter capsulatus*

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UV irradiation and mitomycin C exposure trigger a protease-activity-dependent inhibition of cell division in *Rhodobacter capsulatus*, which begins about 2 h after the treatment is applied. UV irradiation also induces a dose-dependent mutagenesis with a maximal rate between 5 and 10 J m⁻², with increased synthesis of a protein of $M_r$ approximately 30000 between 2 and 3 h after UV irradiation. In addition, *R. capsulatus* has an efficient photoreactivation system that reverses the lethal effects of UV irradiation in the presence of intense visible light.

INTRODUCTION

DNA repair may be carried out in *Escherichia coli* through several pathways such as photoreactivation in the presence of visible light, excision repair, adaptive response to alkylating agents and induction of the SOS response. Photoreactivation seems to be a constitutive process (Hanawalt et al., 1979), whereas the other three mechanisms are inducible (Samson & Cairns, 1977; Kenyon & Walker, 1981; Walker, 1984), although the *uwrA* and *uwrB* genes may be expressed by both SOS-dependent and independent mechanisms (Sancar et al., 1982). The SOS response includes inhibition of cell division, error-prone repair, induction and reactivation of prophages, cessation of respiration, and massive synthesis of RecA protein (for a review see Walker, 1984).

*Rhodobacter capsulatus* (formerly *Rhodopseudomonas capsulata*; Imhoff et al., 1984) belongs to the group of nonsulphur purple bacteria, which can grow phototrophically under anaerobic conditions, or heterotrophically under aerobic conditions. The ability of this group of organisms to exist under two quite distinct physiological conditions, and the subcellular differentiation which accompanies these alternative physiological states, makes a study of its genetics very interesting. Characterization of the response of *R. capsulatus* to UV radiation is of interest. Many bacteria have been shown to induce physiological responses, and/or new proteins, in the presence of agents such as UV radiation which induce the SOS response in *E. coli*. Although a recombination-defective mutant of *R. capsulatus* has been described (Gentner & Wall, 1984), DNA repair systems in phototrophic bacteria have not been studied in detail. For this reason, we have studied the response of *R. capsulatus* after treatment with two DNA-damaging agents, UV radiation and mitomycin C. The results indicate that this bacterium possesses both photoreactivation and SOS-like DNA repair systems.

METHODS

*Bacteria and media.* *Rhodobacter capsulatus* strain B100 has been described previously (Wall et al., 1975). Cultures were grown chemoheterotrophically with shaking at 30 °C in RCVBN medium (Weaver et al., 1975) supplemented with glucose at 0.2% (w/v) and Casamino acids at 0.4% (w/v). Cells treated with mitomycin C were grown in the same medium adjusted to pH 5.5, the optimal pH for the action of this compound (Crooke, 1981). Mitomycin C (sp. act. 4%) and antipain were obtained from Sigma; vitamins, amino acids and mineral salts were...
UV irradiation. Exponentially growing cells were washed by centrifugation, resuspended in RCV medium (Weaver et al., 1975) to a concentration of about \(2 \times 10^6\) cells ml\(^{-1}\) and irradiated in a glass Petri dish in thin layers, with swirling, using a General Electric GY1578 germicidal lamp, at a rate of 0.5 J m\(^{-2}\) s\(^{-1}\) (determined with a Latarjet dosimeter). Biotin, nicotinic acid, glucose and Casamino acids were then added at the required concentrations and the cells were incubated at 30°C. All experiments with UV-irradiated cells, unless otherwise indicated, were done under yellow light or in the dark.

Photoreactivation. After UV irradiation at 40 J m\(^{-2}\), *R. capsulatus* B100 cells were immediately diluted 10-fold in RCV medium and placed about 4 cm from a 125 W tungsten–halogen lamp of a V26 NORIS slide projector for various times, then plated to determine the viable titre. The light source for reactivation was passed through a purple glass filter (EALING 26-330) that transmitted light of wavelengths between 350 and 425 nm. As a control for photoreactivation, a sample of the UV-irradiated cells was placed in the dark to determine the time-dependent evolution of viables in the non-photoreactivating cells. Photoreactivation was done at 30°C in a temperature-controlled room.

Inhibition of cell division. After UV irradiation or mitomycin C addition, cell size was determined with a Coulter counter model ZBI equipped with a 30 μm orifice and with a channelizer (Barbé et al., 1984).

Scoring of mutations for streptomycin resistance. Samples (0.1 ml) of unirradiated or of UV-irradiated suspensions were spread on RCVBN supplemented plates and incubated at 30°C for 18 h before addition of streptomycin to the plates. The antibiotic was added, using soft RCV-agar, to a final concentration of 75 μg ml\(^{-1}\) (Marrs, 1981). After 5 days incubation at 30°C, the total number of streptomycin-resistant (Str\(^r\)) mutants was scored. Colonies scored as mutants were confirmed as Str\(^r\) by their ability to grow at 30°C for 2 d in 2 ml RCVBN-supplemented medium with streptomycin (75 μg ml\(^{-1}\)). The mutation frequencies were calculated as the number of induced mutants divided by the number of surviving cells after adjustment for spontaneous mutants.

Protein synthesis in UV-irradiated bacteria. After UV irradiation at 40 J ml\(^{-2}\), cells were supplemented with glucose at 0.2% (w/v) and all essential amino acids (except cysteine and methionine) at 25 μg ml\(^{-1}\), and the culture was incubated with shaking at 30°C. The cells (1.5 ml) were then pulse-labelled for 15 min at 30°C by the addition of \([^{35}S]\)methionine [10 μg ml\(^{-1}\); 50 μCi (1.85 MBq) ml\(^{-1}\)] at 0, 60, 120 and 180 min. The cells were collected by centrifugation with a microfuge, washed twice with Ringer solution, and resuspended at a 20-fold concentration of the original volume in sample buffer [50 mM-Tris/HCl pH 6.8, 2.5% (w/v) SDS and 5% (v/v) 2-mercaptoethanol]. The samples were then boiled for 5 min and resolved immediately by polyacrylamide slab gel electrophoresis (PAGE).

Electrophoresis and autoradiography. The method of SDS-PAGE employed was that of Laemmli & Favre (1973) except for the use of a 4% (w/v) polyacrylamide spacer gel and 12% separating slab gels which were 1.5 mm thick. The volume of each sample applied to the slab gel was calculated so that an equal number of \(^{35}S\) counts of each sample was applied to each slot. After running, the gels were stained with Coomassie brilliant blue (0.05%, w/v), destained, washed and dried. Labelled proteins were visualized by exposing the dried gels to Kodak X-Omat MA X-ray film at −70°C for 4 d (Laskey & Mills, 1975). The autoradiograph strips were scanned with a Beckman DU-8 spectrophotometer with a gel scanner attachment. To estimate the M\(_r\) of the labelled proteins, the following standard proteins (Sigma) were used: phosphorylase b (97400), bovine serum albumin (66000), ovalbumin (45000), pepsin (34700), trypsinogen (24000) and lysozyme (14300).

RESULTS

Photoreactivation. Exposure of UV-irradiated *R. capsulatus* B100 to visible light resulted in a reversal of the UV-induced killing (Fig. 1). A 10–15-fold increase in viability, raising survival from 2% to about 30% under the conditions used, was routinely seen with 30 min of photoreactivating treatment. Thus *R. capsulatus*, like *E. coli* and many other organisms (Witkin, 1976), has a photoreactivation system for repairing UV-induced DNA damage.

UV-induced mutagenesis. Fig. 2 shows the production of Str\(^r\) mutants by UV irradiation of *R. capsulatus* B100. The rate of increase of UV-inducible mutagenesis was lower at doses higher than 10 J m\(^{-2}\). However, an increase of about 10-fold in the frequency of Str\(^r\) clones was found at a dose of 40 J ml\(^{-2}\).

Inhibition of cell division. UV irradiation at 20 J ml\(^{-2}\) or continuous exposure to 20 μg mitomycin C ml\(^{-1}\) caused a time-related increase in the cell size of *R. capsulatus* B100 (Fig. 3). This filamentation began 2–3 h after the treatment was applied and was greater in the mitomycin C-exposed cells than in the UV-irradiated cells, probably because the former
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Fig. 1. Photoreactivation of UV-irradiated *R. capsulatus*. Strain B100 was irradiated with UV light (40 J m\(^{-2}\)) and exposed afterwards to visible light as described in Methods. ▲, Photoreactivated UV-irradiated cultures; ●, non-photoreactivated UV-irradiated cultures.

Fig. 2. UV-induced mutagenesis of *R. capsulatus*. Strain B100 was irradiated with increasing doses of UV light and the frequency of Str\(^{r}\) mutants was scored as described in Methods.

Fig. 3. Distribution of the cell size of *R. capsulatus* B100 after either UV irradiation at 20 J m\(^{-2}\) (b, d) or mitomycin C addition at 20 μg ml\(^{-1}\) (c, e) in the presence (d, e) and in the absence (b, c) of the protease inhibitor antipain. The distribution of cell size of B100 without any treatment is shown as control (a).
represented a continuous treatment with the DNA-damaging agent whereas UV irradiation was a short-term treatment. Furthermore, this inhibition of cell division after UV irradiation or mitomycin C addition appeared to be related to protease activity, because the addition of 1 mM-antipain, a protease inhibitor, immediately after either treatment prevented cell filamentation (Fig. 3d, e).
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**UV induction of proteins.** The induced amplification of a protein of \( M \), about 30000 was observed after UV irradiation (Fig. 4). The increase in the synthesis of this protein occurred about 2–3 h after UV irradiation (Fig. 4). Densitometric comparison of \(^{35}\text{S}\)methionine-labelled protein bands after SDS-PAGE of extracts from irradiated and unirradiated cells indicated that proteins other than that of \( M \), about 30000 showed relatively minor variations after UV irradiation (Fig. 5).

**DISCUSSION**

Our results show that *R. capsulatus* displays several cellular mechanisms against DNA damage. Thus, after UV-irradiation, *R. capsulatus* cells are able to support a visible-light-dependent repair of UV damage as well as mutagenesis, inhibition of cell division, and also the amplification of a protein of \( M \), about 30000. Furthermore, the inhibition of cell division may be blocked by the addition of the protease inhibitor antipain, which specifically inhibits the expression of several SOS functions in *E. coli* and *Vibrio cholerae* (Meyn et al., 1977; Ghosh et al., 1985). The filamentation of *R. capsulatus* produced by both mitomycin C and UV irradiation may thus be similar to the recA-dependent inhibition of cell division of *E. coli*.

Although the molecular basis for these events is unknown, the UV mutagenesis, amplification of synthesis of some proteins and inhibition of cell division described here are quite similar to elements originally brought together in *E. coli* under the term SOS response (Witkin, 1976). Nevertheless, one obvious difference between the *E. coli* and *R. capsulatus* inhibition of cell division and amplification after UV irradiation is in the time required for maximal expression of these functions. *E. coli* growing in minimal supplemented medium at 37 °C shows maximal expression of these functions about 60 min after irradiation (Salles & Paoletti, 1983; Barbe et al., 1983); *R. capsulatus* growing in minimal supplemented medium at 30 °C induces these phenomena about 120 min after UV exposure. However, this difference correlates with the different generation times for these organisms (40–50 min for *E. coli* and 100–120 min for *R. capsulatus*), extending the similarity between the two systems. It is noteworthy that *R. capsulatus* was mutable by UV radiation; several other bacteria fail to show UV mutagenesis (Gasc et al., 1980; Sedgwick & Goodwin, 1985).

The physiological, genetic and environmental differences between *E. coli* and *R. capsulatus* make further studies on the relationship between the DNA repair genes of both species of considerable interest.

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**REFERENCES**


