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Effect of photoreactivating light on survival of ultraviolet-light-irradiated *Streptomyces lividans* 66

Kristien Mortelmans

SRI International, Menlo Park, CA, U.S.A.

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SUMMARY

Biological systems can repair damage induced in their DNA by ultraviolet light (UV). Most cells contain at least three DNA repair pathways, each of which has a marked effect on UV survival. Excision repair and recombinational (postreplication) repair are light-independent whereas photoreactivation (PR), whether enyzmatic or photochemical, is light-dependent. The specificity of photoreactivation for UV-induced DNA damage allows it to be used as a tool for examining whether premutational DNA lesions are preferred sites for photoreversal; it therefore plays an important role in mutagenesis studies. Evidence is presented here that PR occurs in a time-dependent fashion in three strains of *Streptomyces lividans* 66. The effect appears to be independent of temperature and is observed only when PR treatment is given after UV irradiation. The present experiments do not discriminate between enzymatic and photochemical protection.

INTRODUCTION

Survival of most biological systems exposed to far-ultraviolet (UV) irradiation (230–300 nm) can be increased greatly by posttreatment with near-UV (300–380 nm) or violet and blue (380–500 nm) radiation. This phenomenon is referred to as 'photoreactivation' (PR), and is usually associated with DNA damage induced by far-UV, as very little – if any – PR occurs when the radiation used is from other bands of the electromagnetic spectrum. PR therefore has been defined as "the restoration of ultraviolet radiation lesions in a biological system with light of wavelength longer than that of the damaging radiation" [6].

There are two known mechanisms for PR. The most familiar one is enzymatic PR (EPR) involving a direct light-dependent splitting of UV-induced pyrimidine dimers by the photoreactivating enzyme [15,16]. The reaction is strongly dependent on temperature and radiation dose rate during PR treat-

Correspondence: K. Mortelmans, SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025, U.S.A.

ment. Because of its specificity for UV-induced pyrimidine dimers, EPR has been extensively used to study mechanisms of DNA repair and UV-induced mutagenesis in *Escherichia coli* [17].

In 1965 Jagger and Stafford [8] reported the existence of a second, indirect PR phenomenon in an *E. coli* B strain that could not be photoreactivated under certain conditions. This nonenzymatic PR phenomenon is known as photoprotection. It most likely involves an initial photochemical reaction, followed by a series of steps (some of which may be enzymatic) that ultimately lead to light-independent 'dark' DNA repair [18]. In contrast to EPR, photoprotection is independent of the dose rate of the protecting radiation treatment and nearly independent of temperature. Photoprotection also requires much higher doses of visible light than does EPR.

UV radiation is one of several important mutagenic agents used in the process 'mutagenesis and random screening' for selecting improved industrial strains [14]. Although important advances have recently been made in gene-cloning and genetic recombination by protoplast fusion in the economically important streptomycetes, little is known about mechanisms of DNA repair and mutagenesis in these prokaryotic organisms. Yet, it is through our understanding of DNA repair and mutation mechanisms that mutagenesis procedures can be optimized for the selection of desirable mutants.

Studies have been undertaken on mechanisms of DNA repair in *Streptomyces lividans* 66, which has largely replaced *S. coelicolor* as the standard strain for genetic studies as well as the host strain for gene-cloning experiments involving both plasmid and phage vectors. *S. lividans* offers several advantages over its close relative *S. coelicolor* which is still the best characterized streptomycete [4]. Wild-type *S. lividans* 66 carries two well-characterized self-transmissible plasmids, SLP2 and SLP3, is suitable for transformation experiments as it is not known to restrict DNA from any other streptomycete, and has a slightly faster growth rate and more copious spore production than *S. coelicolor*.

This paper describes results of PR experiments to determine whether UV irradiation induces DNA lesions that can be photoreactivated in *S. lividans* strains.

MATERIALS AND METHODS

Strains and Media

Three related *S. lividans* 66 spore-producing strains were used [4]. Strains JI1326 ($SLP2^+$, $SLP3^+$), and TK64 ($SLP2^-$, $SLP3^-$, *pro-2, str-6*) were obtained from Dr. Stanley Cohen, Stanford University. Strain TK54 (*his-2, leu-2, spec-1*) was obtained from the Waksman Institute, Rutgers University, New Jersey (Workshop, June 11–13, 1986. Molecular Biology and Genetics of Streptomycetes). The abbreviations *pro, his, leu* denote auxotrophies for proline, histidine and leucine; *str* and *spec* indicate resistance to streptomycin and spectinomycin, respectively. Sporulation medium R2YE (also called R5) and minimal agar medium (MM) were prepared as described by Hopwood et al. [3]. Minimal agar medium was supplemented with required amino acid(s). Yeast complete agar medium (YC) consisted, per 1 liter, of: 0.5 g of $MgSO_4 \cdot 7H_2O$, 1.5 g of KH_2PO_4, 4.5 g of $(NH_4)_2SO_4$, 3.5 g of peptone, 5 g of yeast extract, 20 g of dextrose, 20 g of agar. Nutrient agar (NA) plates were prepared with Oxoid blood agar base (CM55).

Spore collection and storage

The spores were harvested from several R2YE plates after 5 days' incubation at 30°C. The spores from each plate were collected in 10 ml of sterile saline and passed through a syringe packed with sterile glass wool to retain the mycelial fragments. The filtered spore suspensions were centrifuged for 10 min at 7000 rpm. Each pellet was resuspended in 2 ml of 20% (v/v) glycerol. Prior to freezing at −20°C, the spore suspensions from the different plates were pooled and dispensed into small cryogenic tubes in 1-ml aliquots. For each experiment, frozen spore suspensions were allowed to thaw at room temperature. The unused portions of the thawed spore suspensions were immediately placed back in the −20°C freezer.

UV-survival curves

Spore suspensions were serially diluted in sterile distilled water. Drops, of volume 0.01 ml, from each dilution were delivered to MM agar plates supplemented with required amino acid(s), YC or NA plates. This procedure allows for the plating of spores from up to six different dilutions in triplicate on one plate, and only one plate per UV dose is needed [13]. When the drops had dried, the plates were exposed to UV doses of 0, 50, 100, 150, and 200 J/m^2. The UV source was a 15-W General Electric germicidal lamp with peak output at 254 nm at a fluence of approximately 0.6 J/m^2/s, as measured by an IL570 photometer (International Light, Newburyport, MA 01950). Colony numbers were counted in the drop areas after approx. 2 days' incubation in the dark at 30°C. Extreme care was taken to count colonies when their sizes were small enough so they would not overlap. Irradiation and subsequent procedures were carried out under yellow light as a precaution against possible PR.

PR

PR studies were carried out by preparing two sets of plates as described above. Immediately after UV treatment, one set of plates was exposed to light from three white fluorescent light tubes (General Electric Watt-Miser 35 F400W-RS-WM cool white) at a distance of 75 cm for various amounts of time, unless otherwise indicated. The other set of plates was wrapped in aluminum foil and placed next to the exposed set in order to keep treatment conditions nearly identical. The temperature during the white-light treatment was approximately 18°C. Colonies were counted after about 2 days of incubation at 30°C.

RESULTS

Posttreatment with photoreactivating light

Initial experiments were performed on YC plates with strain TK54. Spores were exposed to UV at doses of 0, 50, 100, 150 and 200 J/m^2 as described in Materials and Methods. A slight increase in survival was observed when PR treatment was given for 0.5 h (data not shown). When exposure time to visible light was extended from 0.5 to 1 h, or 1.5 h, a time-dependent increase in survival was observed; an increase in PR was still observed after 24 h of exposure to visible light. These results are presented in Fig. 1.

Having established that *S. lividans* strain TK54 possesses PR properties, PR studies were performed on wild-type strain JI1326 carrying plasmids SLP2 and SLP3, and TK64, which is auxotrophic for proline and resistant to streptomycin. Survival of the spores of the two strains after exposure to UV doses of 0, 50, 100, 150 and 200 J/m^2 on YC medium, with and without a 24-h PR treatment, was nearly identical to that of strain TK54 (see Fig. 1 for TK54 survival data).

Effect of medium on UV-survival and PR

The three *S. lividans* strains were used to establish whether medium composition affects their UV-survival and extent of PR, as has been reported for other biological systems [7]. Spores were plated on YC, NA, and MM medium and irradiated at UV doses of 0, 50, 100, 150, and 200 J/m^2, as described in Materials and Methods. One set each of the plates was exposed to PR light for 24 h at 18°C, and the other set was kept in the dark. UV dark survival, determined after additional incubation at 30°C for about 2 days, was reduced on MM medium compared with the near identical survival on YC

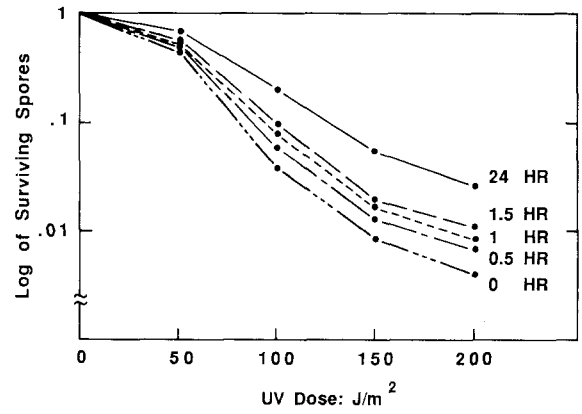

Fig. 1. UV-survival of *S. lividans* TK54 spores with post-PR treatment at the indicated exposure times of 0, 0.5, 1, 1.5 and 24 h.

and NA medium. However, PR treatment was more effective at increasing the extent of enhanced UV-survival on MM medium than when YC or NA medium was used. Fig. 2 presents representative survival data with and without PR treatment with strain TK54 on MM and NA medium.

Pretreatment with PR light

Photoprotection is observed in some biological systems when PR treatment is given before as well as after far-UV exposure, while EPR is observed only after UV irradiation [7]. In this study eight sets of YC plates were prepared with spores of *S. lividans* strain TK54, as described in Materials and Methods, for determining UV survival. Prior to UV irradiation at doses of 0, 50, 100, 150, and 200 J/m^2, one set each of the plates was exposed to PR light for 0.5, 1, 1.5 and 24 h, while the other corresponding set was wrapped in aluminum foil. UV irradiation of both the PR-treated and untreated sets of plates was carried out promptly at each of the indicated PR time points. No difference in survival was observed between the untreated and PR-treated spores (data not shown).

Effect of temperature on PR

EPR is dependent on temperature, whereas photoprotection, believed to be an initial photochemical reaction, exerts its effect nearly independently of temperature. To study temperature effects on PR, two sets of YC plates were prepared with spores of *S. lividans* strain TK54. UV irradiation was carried out on two sets of plates at doses of 0, 50, 100, 150 and 200 J/m^2. One set of plates was immediately wrapped in aluminum foil and placed alongside the other set of plates, which was exposed for 0.5 h to two Sylvania 35-W F15T8/CW fluorescent light tubes in a room maintained at 37°C. Survival was determined after additional incubation in the dark for 2 days at 30°C. The same slightly enhanced survival level was observed as is presented in Fig. 1 when PR treatment was carried out at 18°C.

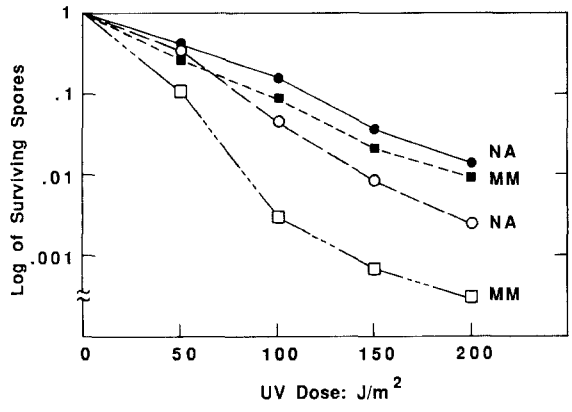

Fig. 2. Effect of NA and MM on survival of *S. lividans* TK54 spores with 24-h PR treatment (●, ■) and no PR treatment (○, □).

DISCUSSION

Spores of three *S. lividans* 66 strains were examined for the ability to photoreactivate UV-induced DNA damage by measuring levels of survival under different experimental conditions. The results indicate that at least one PR mechanism is operating in the *S. lividans* strains examined. The PR effect is time-dependent, with an enhanced UV survival still evident after 24-h exposure to visible light, as shown in Fig. 1 for strain TK54. A similar effect was observed with strains JI1326 and TK64. At this time no studies have been performed to optimize PR treatment as far as exposure time is concerned. Instead, a 24-h exposure to PR light was arbitrarily selected as standard treatment, since no adverse effects were observed on spore viability during this prolonged PR treatment.

The slight protective effect observed in the *S. lividans* strains after 0.5-h PR treatment is similar to that observed in wild-type *S. coelicolor* A3(2) by Harold and Hopwood [2], who reported heterogeneity with respect to photoreactivity in wild-type *S. coelicolor* [2]. In the same study the authors reported a major PR effect in wild-type *S. coelicolor* K673. Photoprotection was implicated as the mechanism involved in the low-level protection in *S. coelicolor* A3(2), whereas a presumed EPR mechanism was implicated in the extensive PR effect observed in wild-type *S. coelicolor* K673. An action spectrum for PR of UV-irradiated spores of *S. griseus* (ATCC

No. 3326) and an *E. coli* B/r strain was established by Kelner in 1951 [9]. Peak activity was observed at or near 436 nm in *S. griseus,* compared with 375 nm in *E. coli* B/r. These observations indicate that the chromophores absorbing the photoreactivating energy are not the same in the two species.

Fig. 2 reveals an increase in UV killing when *S. lividans* spores were plated on MM plates, compared to that when YC was used. These results are supportive of those published by Jagger [7], who demonstrated a more pronounced survival of UV-irradiated *E. coli* B when plain (100%) NA medium was used as compared with a minimal salt agar supplemented with 1.5% nutrient agar, which is considered to be a poor growth medium for this organism. No difference, however, was observed in the survival of UV-irradiated *S. typhimurium hisG46* on MM enriched with 1% nutrient broth and on plain NA medium [13].

The survival data presented in Fig. 2 also indicate a larger PR effect on MM than when YC was used. These results are consistent with those reported by Jagger [7], who demonstrated more PR when UV-irradiated *E. coli* B were plated on minimal salt agar containing 1.5% nutrient agar than when they were plated on plain (100%) nutrient agar. Differences in medium composition are also known to affect PR in cultured human cells. Mortelmans et al. [12] reported a unique medium-dependence to demonstrate PR in cultured human fibroblasts; however, the study did not allow differentiation between EPR and photoprotection.

Certain strains of *E. coli* also exhibit dark reactivation after UV exposure when plated on medium containing catalase or iron salt with an additional slightly light-enhanced survival [10]. The presence of iron salt in the MM medium used in the studies reported here could be a causative element in the enhanced PR of *S. lividans*. However, there is no conclusive evidence to support such a hypothesis fully. Also, there is no evidence for a medium-dependent dark reactivation phenomenon in *S. lividans*.

PR was observed only when PR treatment was given after UV irradiation of the spores. In some biological systems, however, photoprotection is observed when PR treatment is administered before and after UV treatment [8]. On the other hand, PR of the *S. lividans* strains was independent of temperature, which is supportive evidence for the presence of a photoprotective mechanism.

The similarity in UV survival with and without PR treatment among the three *S. lividans* strains is not surprising considering their relatedness [4]. Plasmids SLP2 and SLP3 housed by strain JI1326 apparently do not offer a UV protective effect that has been reported for a number of colicin and R plasmids in *E. coli* and *S. typhimurium* [5,11].

The work presented here provides evidence that visible light treatment enhances UV survival in three *S. lividans* strains. However, the present experiments do not discriminate between EPR and photoprotection. The extent of UV survival and PR are medium-dependent. The demonstration that PR occurs in *S. lividans* strains is an important finding, because the specificity of PR for UV-induced DNA damage allows it to be used as a tool for examining whether premutational DNA lesions are preferred sites for photoreversal. Also, the present findings warrant the use of subdued non-photoreactivating light when performing UV-irradiation experiments with *S. lividans* 66 strains.

As a final note, in this study advantage has been taken of the copious spore production of the three *S. lividans* strains. The use of spores for genetic studies has also been reported for *S. coelicolor* [1]. For the experiments reported here, a large stock supply of spores was prepared that allowed the use of the same batch of spores over a period of several weeks. Prior to the PR work, experiments were carried out to determine whether storage of the spores in 20% glycerol at $-20°C$ would have an effect on their stability and viability. For this purpose, spore suspensions were examined at weekly intervals over a period of 10 weeks for plating efficiency and UV survival. No adverse effects on viability of the spores and UV survival were observed, even when frozen spore suspensions were thawed and refrozen up to 10 times during this period.

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