Deoxyribonucleic Acid Repair Capacities of *Neisseria gonorrhoeae*: Absence of Photoreactivation

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No difference in survival was observed when ultraviolet-irradiated gonococcal cells were subsequently incubated in the dark or exposed to photoreactivating light. This observation indicates that photoreactivation is absent in *Neisseria gonorrhoeae*.

Direct photoreactivation is mediated by a single enzyme whose only known substrates are pyrimidine dimers in polynucleotides (12, 13, 15, 16). The enzyme has been found in essentially every phylum in which it has been investigated (3, 14) except bacteria that have the ability to be competent, that is, to bind nucleic acid in a form resistant to nuclease (22). *Neisseria gonorrhoeae*, which is competent (17), has not been investigated for photoreactivation. Thus, we undertook this study.

Strains WLPI (*met*, obtained from F. E. Young of the University of Rochester, Rochester, N.Y.), FA19 (wild type, obtained from P. F. Sparling of the University of North Carolina at Chapel Hill), or FA248 (*met pro*, obtained from P. F. Sparling) were inoculated into GCK liquid media (7) and grown to mid-exponential phase. Cells were then exposed to varying fluences of UV irradiation. After exposure, cells were immediately exposed to a source of photoreactivating light for 60 min. Two Westinghouse 15W F15T8 black-light-blue (BLB) fluorescent lamps were initially used as the photoreactivating light source since they emit 97% of their radiation at wavelengths between 300 and 400 nm, with a peak wavelength occurring at 366 nm (5). Wavelengths below 300 nm were eliminated by use of a 6.4-mm-thick piece of window glass. Unless stated otherwise, all exposure to UV irradiation (254 nm) and plating procedures were done under gold fluorescent lamps which emit wavelengths only above 500 nm, thus avoiding any photoreactivation of cells before exposure to photoreactivating light.

As Fig. 1 indicates, there was no difference in survival of UV-irradiated cells of strain WLPI that were exposed to photoreactivating light versus UV-irradiated control cells not exposed to photoreactivating light. Similar results were obtained with strains FA19 and FA248 (data not

shown). Furthermore, survival of cells not exposed to photoreactivating light compared with cells exposed to photoreactivating light for 60 min was 5.2×10^7 and 4.9×10^7 colony-forming units per ml, respectively. Thus, photoreactivating light was neither beneficial nor detrimental to cell survival.

To insure that the experimental conditions employed to photoreactivate the gonococcal cells were adequate, both positive and negative controls were utilized. *Escherichia coli*, the positive control, has an efficient system of photoreactivation (9). The action spectrum of the *E. coli* photoreactivating enzyme extends from 300 to 500 nm with a maximum near 380 nm (6). *Bacillus subtilis*, the negative control, lacks a photoreactivation system (8). There was a marked increase in survival of UV-irradiated *E. coli* cells that were subsequently exposed to photoreactivating light compared with unexposed cells, but there was no appreciable enhanced survival of UV-irradiated *B. subtilis* cells that were subsequently exposed to BLB compared with unexposed control cells (data not shown).

Since human leukocyte photoreactivating enzyme has been shown to have an action spectrum for photoreactivation that extends to a wavelength of 600 nm (21), the possibility was investigated that the action spectrum for the gonococcal photoreactivating enzyme might also be different from that for the *E. coli* and other photoreactivating enzymes, so a second source of photoreactivating light was utilized. This source was "daylight" fluorescent lamps which emit a much broader range of wavelengths (375 to 750 nm with a maximum at 575 nm) than BLB (5). Mid-exponential-phase cells of WLPI were exposed to varying fluences of UV irradiation and subsequently subjected to a 60-min exposure to two Westinghouse 15-W F15T8 flu-

FIG. 1. Mid-exponential-phase cells of strain WLPI were centrifuged at $10,000 \times g$ for 10 min at 4°C , suspended in Spizizen salts (19), and diluted to a final concentration of 4×10^7 to 8×10^7 colony-forming units per ml. A 10-ml portion was placed in a 100-mm glass petri plate and irradiated with a Sylvania G8T5 germicidal lamp (254 nm) at a flux of 0.9 W/m^2 for various time periods. Samples of 2 ml each were placed in a glass petri dish and incubated at room temperature in the dark (○) or under photoreactivating light (■) for 60 min. The plates were placed 12 cm under the BLB fluorescent light source.

orescent lamps. Again, photoreactivation did not occur (data not shown).

Sutherland and Sutherland have suggested that only red lights which emit no wavelengths below 600 nm should be used as safelights if the action spectrum of the photoreactivating enzyme extends past 500 nm or if the action spectrum is unknown (20, 21). Hence, the above experiment was repeated using daylight fluorescent lamps as the photoreactivating light source and a red incandescent lamp as the safelight. The results were the same as those above.

Since stationary-phase cells can have a higher concentration of photoreactivating enzyme than exponentially growing cells (1, 21), the photoreactivating capabilities of stationary-phase cells of WLPI were investigated. Stationary-phase cells were exposed to varying doses of UV irradiation, with subsequent exposure to BLB for 60 min. There was no significant difference in survival of UV-irradiated, stationary-phase cells exposed to photoreactivating light versus irradi-

ated, stationary-phase cells not exposed to photoreactivating light (data not shown).

Studies have shown that *Neisseria meningitidis*, also a competent organism (2), and *N. gonorrhoeae* are closely related to each other since they share 72 to 80% of their base sequences (4, 10, 11). Hence, the photoreactivation capabilities of three clinical isolates of *N. meningitidis* were investigated. Mid-exponential-phase cells were UV irradiated and subsequently exposed to BLB as described in Fig. 1. The results obtained were similar to those of Fig. 1 (data not shown).

Thus, the results of this investigation clearly show that *N. gonorrhoeae*, and apparently *N. meningitidis*, join the ranks of other investigated transformable organisms in lacking a photoreactivation system of DNA repair. The significance of this phenomenon is being investigated.

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