The Effects of Ultraviolet Irradiation on a Coccoid Blue-green Alga: Survival, Photosynthesis, and Photoreactivation

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Received June 7, 1968.

Abstract. The effects of UV irradiation (254 m\(\mu\)) on a coccoid blue-green alga *Agmenellum quadruplicatum*, Strain PR-6, have been examined in terms of the survival curve and measurement of short time photosynthetic rates. From study of survival evidence has been found for a strong photoreactivation centered near 430 m\(\mu\). Measurements of photosynthetic rate suggest that there is a correlation between decay of photosynthesis and survival after UV exposure. The UV induced decay in photosynthetic activity is reversed by the identical photoreactivation conditions that increase the survival level. The photosynthetic data are interpreted as demonstrating a photoreactivation of photosynthesis in blue-green algae.

The effects of radiation in the 220 to 300 m\(\mu\) range on blue-green algae are not well known. Halldal (3) has noted the effects of UV irradiation on photosynthesis in *Anabaena* sp. Several reports have appeared comparing characteristics of ultraviolet resistant and wild-type strains of blue-green algae (7, 12). Photoreactivation of UV damage of the blue-green algal virus LPP-1 within the host cell has been described (18). Evidence has also been presented that *Plectonema boryanum* contains photoreactivating enzyme when tested in the *Hemophilus influenzae* transformation assay (17). The present report deals with the nature of survival and photosynthesis in *Agmenellum quadruplicatum*, Strain PR-6, a coccoid blue-green alga after ultraviolet irradiation (254 m\(\mu\)). Both the survival level and the decay in photosynthesis after UV exposure are markedly influenced by a photoreactivation. An abstract of an early part of this work has previously appeared (14).

Materials and Methods

*Agmenellum quadruplicatum*, Strain PR-6, hereinafter referred to as PR-6, is an isolate of this lab. It was routinely grown batch-wise in liquid culture using the test tube culture method of Myers (8) at 39° with 1% CO\(_2\)-in-air continuously bubbled through the tubes. Illumination was provided by four 30 w cool white HO fluorescent lamps (two on each side) 7.5 cm from the tubes. At harvest the cell volume was approximately 1 mm\(^3\)/ml.

*Chlorella pyrenoidosa* (Emerson strain) was similarly grown but on Knop's medium at 25° (10).

Suspensions used for UV irradiation and measurement of photosynthesis were handled in the same manner as for PR-6.

In the plating experiments with PR-6 the test tube culture baths were modified to hold 10 Corning flasks (No. 5100, 125 ml). A 15 ml base layer of medium containing 1% Difco agar 0140, was placed in the flask. A 0.1 ml aliquot of the cell suspension containing 100 to 300 cells was placed on the agar surface, then 1 ml of agarized medium at 44° was added and mixed with the suspension. Illumination was provided by a linear band of seven 60 w tungsten bulbs 15 cm from the flasks. The flasks were incubated at 39° with CO\(_2\) supplied by diffusion. The medium for both flask and liquid growth was slightly modified ASP-2 (16) containing 2 \(\mu\)g/1 vitamin B\(_{12}\). A more complete discussion of the factors involved in the quantitative single-cell plating of coccoid blue-green algae has been published (16).

To make the cell suspension used for measuring survival or photosynthesis, an exponentially growing culture of PR-6 was centrifuged, resuspended in fresh growth medium and diluted to approximately 1 \(\times\) 10\(^5\) cells/ml. Six ml of cell suspension was placed in an open petri dish (50 mm) and irradiated at a distance of 48 cm from a GE G15T8 lamp. In order to make all the UV dosages as equivalent as possible, all doses were given as a 1 min exposure period (17.5 ergs mm\(^{-2}\) sec\(^{-1}\)) with a 10 to 15 sec intervening dark period, until the total dose desired had been reached. Thus, continuous irradiation periods greater than 1 min were interrupted in the same fashion as irradiation periods in which samples were periodically removed. The suspension was continuously stirred during the irradiation period. To prevent photoreactivation effects all operations were done under a safelight consisting of a tungsten lamp covered by Rohm and Haas Plexiglas, transparent colored sheet No. 2422, 0.125 inch thick.

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1 This investigation was supported by National Science Foundation Grant GB 4721X.
Immediately preceding irradiation the cell suspension received low intensity illumination from the safelight.

The wavelengths used for photoreactivation were isolated using Bausch and Lomb second order interference filters with appropriate Corning blocking filters. The light sources were Standard 500 Junior Projectors with the heat filters removed. The cell suspensions were held at 39° with 1% CO₂-in-air bubbled through the tubes during the photoreactivation period.

Photosynthesis was measured with a Gilson Medical Electronics Clark-type electrode (OX700) and water-jacketed cell (OX705). Changes in electrode current were amplified on a Keithley 150A Microvolt-Ammeter and recorded on a Brown Recorder. Carbon dioxide was supplied in a 5 min gassing period as 1% CO₂-in-nitrogen prior to closing the electrode chamber. The choice of 1% CO₂-in-nitrogen in place of 1% CO₂-in-air was made to prevent the build-up of high O₂ concentrations during photosynthesis in the sealed electrode chamber. Illumination was provided by the above projector system with appropriate filters.

All light intensity measurements were made with a Kipp and Zonen CA-1 thermopile and Keithley 150A Microvolt-Ammeter. The calibration given by Kipp and Zonen was checked using a standard lamp. The energy values given were those incident upon the cell container.

Results

Survival. Figure 1 shows the survival curves for PR-6. Curve 1 is the survival for cells which were irradiated for the doses shown, plated, and immediately returned to growth conditions. Note that in this case the tungsten illumination used for growth was filtered through a sharp-cut yellow filter, Rohm and Haas Plexiglas No. 2422. Curve 2 is the same experiment except that the tungsten illumination used for growth was not filtered through the sharp-cut yellow filter. Curve 5 was constructed from the data in figure 2 which shows the rate and extent of photoreactivation in monochromatic light (430 μm) as a function of UV dose. The family of curves relating rate and extent of photoreactivation at 395 and 459 μm were similar in general characteristics to those shown in figure 2. However, the slight suggestion of a lag period in recovery in the 430 μm curves at the longer UV irradiation times was much more evident in the 395 and 459 μm curves. The lag in photoreactivation at 395 and 459 μm began and then increased as the survival curve departed from 100% recovery. The curves in figure 3 relate the rates of photoreactivation after a 2.5 min UV irradiation to the wavelength used for photoreactivation.

As basis for discussion let curve 1 of figure 1 be considered as the "normal" response curve of PR-6 to UV irradiation.

Curve 1 is not very different from the curve for E. coli B/r as found by Kelner (4). Thus in the absence of photoreactivating wavelengths in the illumination used for growth, the UV sensitivity of a cocccoid blue-green is much like the heterotroph, E. coli B/r. This may be a coincidence but it is curious that 2 such different organisms should give similar survival curves.

Secondly, the difference in survival level between curve 1 and curve 2 (white light) represents a much larger dose reduction, L/D, than found by Kelner for E. coli B/r of 2.5 (4). For PR-6 at 10⁻¹ survival L/D = 5, for 10⁻³ survival L/D = 4.5. In a monochromatic beam, curve 5, L/D is even higher. Clearly, then, photoreactivation in PR-6 is an appreciable effect which allows 100% recovery of all cells (compare curves 1 and 5 of fig 1).
Figure 2, Time course of photoreactivation at 430 μm, for organism PR-6, as a function of UV dose. Cells irradiated, placed at 39° in photoreactivating beam, bubbled with 1% CO₂ in air, and plated at times shown. Illumination for growth filtered through Plexiglas No. 2422. From the left the UV dosages were; 3150, 5250, 7350, 9450, 10,500, and 11,550 ergs/mm², respectively. Photoreactivating beam consisted of B and L 430 μm interference filter plus Corning C.S. 4-71 filter, incident intensity 600 μw/cm². The X at the end of each curve is the survival level of a flask plated at the end of the experiment and placed under the same growth conditions as above but without the yellow filter. The point was taken as the maximum degree of photoreactivation after the given UV dose and was the value used to construct the curves in figure 1. The dashed curves are recovery at 24.5°, 3150, and 7350 ergs/mm² UV dose.

Figure 3 represents the rate of photoreactivation as a function of wavelength after a given UV dose. The data were taken from an older series of experiments and are not viewed as constituting a precise action spectrum. Nevertheless, they track well with the data in figure 1 and suggest an action spectrum in a similar region as found for *Streptomyces* by Kelner (5). Considering the plethora of blue light effects in algae and other systems (e.g. 6) a more precise examination is indicated, especially in the region below 395 μm. Of special interest is the finding here, also seen by Wu et al. (18), that photosynthetically active regions have no discernible effect. This would tend to rule out processes such as ATP production, carbon metabolism, formation of reduced coenzymes, as directly useful for repair in the absence of photoreactivating wavelengths.

Photoreactivation was not influenced by bubbling the cell suspension during photoreactivation with...
argon (prepurified grade) or 1% CO₂-in-nitrogen. When UV treated cells were photoreactivated directly in the electrode chamber with a 430 mμ beam the gas exchange seen was the same as with an unirradiated suspension. Apparently there is no gross O₂ requirement for photoreactivation. A 60 min dark period between UV irradiation and exposure to photoreactivation likewise had no effect. Lowering the temperature to 24.5° slowed the rate of photoreactivation (fig 2).

The foregoing observations on photoreactivation in PR-6 are completely supported by earlier work in this laboratory with the fresh-water coccolid Anacystis nidulans, Tx 20. Hence a more general interpretation seems warranted; that there is in blue-green algae a very strong photoreactivation, centered near 430 mμ.

One purpose in examining UV irradiation in blue-greens was its possible use as a mutagenic agent. It should be pointed out that in contrast to a series of visible colony mutations easily seen on plates after treatment of PR-6, A. nidulans and other blue-green algae with N-methyl-N′-nitro-N-nitrosoguanidine (15); no such colonies have ever been found among the survivors of many UV experiments.

Photosynthesis. Figure 4 shows the decay in photosynthetic rate in PR-6 after UV irradiation. The general conditions, cell suspensions used, and irradiation dose were exactly the same as for a survival experiment. The gradual decay in photosynthetic rate rather than an immediate stoppage of photosynthesis is typical of previous results with Chlorella (11). Insofar as the log scale of survival can be compared to a short time measure of photosynthetic rate it is considered that there exists a close correlation between curve 1 of figure 1 and the decay curves in figure 4. That is, the same dosages that caused decreased survival also killed photosynthesis. When the photosynthetic rate of UV irradiated cells was followed for a longer time than shown in figure 4, O₂ evolution ceased and O₂ uptake typical of respiration ensued. The cessation of photosynthesis was judged permanent, dark periods and further light periods failed to reveal any recovery of O₂ evolution.
The rate of photosynthesis for PR-6 control cells, upper curve, figure 4, of 42 \( \mu \text{mol} \text{hr}^{-1} \text{mm}^{-2} \) of cells is a little lower than would be anticipated from previous work with another coccolid, *Anacystis nidulans* (9). However, the same results with control cells or UV irradiated cells were obtained in ASP-2 plus NaN\(_3\), in 1% CO\(_2\)-in-air, or at lower intensities (fig 7).

Figure 5 demonstrates that after 7 min UV irradiation and the same photoreactivation time at 430 m\( \mu \) needed to bring the survival level (as measured by single cell-count) back to 100% recovery, photosynthesis also immediately and completely recovered.

The data of figure 5 were purposely taken under a light saturation condition approaching maximum. Therefore the recovery of photosynthesis after UV irradiation and the photoreactivation period is complete, as seen in a short time measure of photosynthesis.

The data then suggest that a major part of the UV damage in PR-6, up to a dose of 7350 ergs mm\(^{-2}\) sec\(^{-1}\) (7 min exposure), is occurring in some part of the photosynthetic machinery and that photoreactivation is immediately repairing this damage. The data in contrast to other systems (13) do not implicate DNA unless a heretofore unsuspected direct connection exists between the photosynthetic machinery and DNA in blue-green algae. Further, after the photoreactivation period, cells of PR-6 were capable of growing without any lag. As measured in liquid culture the growth rate after UV exposure and photoreactivation was indistinguishable from that of a control culture (fig 6). It was also a common observation in this work that the survivors after UV treatment and photoreactivation showed no growth lag as evidenced by early comparison of colony size with control flasks.

Rigorous proof of the idea expressed above that UV damage and photoreactivation in PR-6 do not involve DNA must obviously await identification of the molecular species involved. Examination of the characteristics of the decay of photosynthesis after UV irradiation have so far not allowed any localization of the damage. When cells were kept in darkness after the UV exposure the decay curve for photosynthesis remained constant for at least 3 hr. Figure 7 shows what is considered to be a characteristic of the UV damaged photosynthetic machinery, that is, an increased sensitivity (more rapid decay) as a function of light intensity, perhaps a photooxidation. However, there has so far been no clear evidence that the light intensity-decay relationship has any pronounced wavelength dependency.

While not carefully examined here, the rate of respiration was routinely measured at the end of
Each photosynthetic run. Suffice it to say that in agreement with results with Chlorella (11) immediate effects on respiration were not seen. The respiration rate even after 10 min UV dose (10,500 ergs/mm²) was not in a short time measurement different from control cells.

Finally, by way of comparison, cells of Chlorella pyrenoidosa at a suspension density equivalent to PR-6, and under the same conditions as used for PR-6, were exposed to UV irradiation. Figure 8 shows that at approximately twice the UV dose for PR-6, photosynthesis decayed with similar kinetics. No evidence was found with Chlorella that monochromatic light at 430 μm, broad regions in the blue, or selected regions from 550 μm to 700 μm, had any immediate photoactivating effect on the UV damage to the photosynthetic machinery.

Discussion

The blue-green algae are an ancient group, which must have early developed means to cope with harmful radiation; it is therefore not surprising to find in them a powerful photoreactivation system. The photoreactivation system in PR-6 can completely negate a much larger UV dose (254 μm) than in E. coli. Extrapolation of curve 1 of figure 1 suggests that photoreactivation can bring all cells back to normal from a survival level which must approach zero. Perhaps it was just such a capability which enabled them to develop in the ancient environment (1).

The notion advanced here that the same UV doses that affect survival also damage the photosynthetic machinery of PR-6 is clearly true. This may be an important point with blue-green algae since the nucleoplasm is within and probably in most cases shielded by the photosynthetic lamellae. The correlation between the survival curve (curve 1, fig 1) and the loss of photosynthetic capacity (fig 4) suggests that the cause of cellular death is the photosynthetic damage.

Taken as a whole the results on photosynthetic decay after UV, photoreactivation of photosynthesis, and lack of growth lag, are hard to interpret as resulting from phenomena occurring in DNA, at least, up to the point of complete survival (7 min UV dose), curve 5, figure 1. Rather, the data suggest to me that within this UV dose range the chromophore for UV damage and the action of photoreactivation is in the photosynthetic machinery. Secondary phenomena, perhaps with DNA, may be involved at the point at which the survival curve departs from 100% recovery. It remains for future work to determine the type of cellular damage operating in this region of the survival curves.

It is an obvious extrapolation of the data to suggest that the difference between PR-6 and Chlorella, with respect to photoreactivation, lies in the tetrapyrrole, phycocyanin. There may be basis for this idea in the fact that in the bile pigment series differences in conjugation between the pyrrole residues (2) could allow for the complete series of molecular species required for absorption of UV at 254 μm, photoreactivation around 430 μm, and photooxidation sensitivity around 620 μm. It should be noted that only in blue-green algae does the combination of procaroytic cell-type with a tetrapyrrrole occur.

The fact that photosynthesis and photoreactivation are essentially competing reactions, one leading to death, the other to repair, is supported by the present interpretation of the survival curves 2 and 5 in figure 1. The differences between curves 3, 4, 5 of figure 1 are also interpreted in this manner. That is, at any wavelength at which photoreactivation is not driven at a sufficient rate to repair photosynthesis then irreversible damage to the photosynthetic machinery occurs and death ensues.

Acknowledgments

I am greatly indebted to Jo-Ruth Graham and Dr. Jack Myers for the culture of Chlorella pyrenoidosa, for aid in calibration of the thermopile, and for the suggestion of and wise council in the use of the electrode. I am also indebted to Dr. E. L. Powers for his comments and criticisms of the manuscript. The competent technical assistance of Mrs. Rita O'Donnell is gratefully acknowledged.
Literature Cited