CHLOROFLEXUS AURANTIACUS AND ULTRAVIOLET RADIATION: IMPLICATIONS FOR ARCHEAN SHALLOW-WATER STROMATOLITES

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Abstract. The phototrophic growth of *Chloroflexus aurantiacus* under anoxic conditions was determined as a function of continuous UV irradiance. Cultures grown under an irradiance of 0.01 Wm^{-2} exhibited a slightly depressed yield over the non-irradiated control. Yields decreased further with increasing irradiance. Inhibition was severe at an irradiance of 0.66 Wm^{-2}. Growth of *E. coli* cultures was severely depressed at UV-C irradiances that permitted good growth of *C. aurantiacus*. Low levels of Fe^{3+} provided a very effective UV absorbing screen. The apparent UV resistance of *Chloroflexus* and the effectiveness of iron as a UV-absorbing screen in sediments and microbial mats are suggested to be likely mechanisms of survival of early phototrophs in the Precambrian in the absence of an ozone shield.

1. Introduction

The presence of microfossils of filamentous prokaryotic microorganisms from 3.5 Ga ago (Schopf and Walter, 1983) and the existence of stromatolites of similar age (Walter, 1983) provide compelling evidence for the presence of well-developed microbial mat communities in shallow water environments during the Earth's early history. It appears that these organisms grew and diversified at the interface of sediment and water. This environment would have permitted photosynthesis but would also have resulted in intermittent exposure to the atmosphere (Walter, 1983). Oxygen levels sufficient for the formation of an effective ozone shield were probably not established until the Proterozoic Eon (2.5 to 0.55 Ga ago) (Kasting, 1987). Consequently, organisms in a shallow water habitat were most likely subjected to relatively high levels of ultraviolet (UV) radiation. If one accepts the fossil evidence that some early stromatolites were formed in a shallow water environment, then one must seek out the conditions that permitted life to exist in the presence of high levels of UV radiation.

Ozone strongly absorbs radiation in the wavelength range of 220 to 300 nm. Radiation of wavelengths less than 280 nm (190–280 nm) is designated as far UV or ultraviolet C (UV-C) radiation (Robberect, 1989). UV-C is more damaging to cells than UV-B (280–320 nm) and UV-A (320–400 nm) because DNA strongly absorbs radiation in this wavelength range. DNA mutations and lethal cell damage

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occur when UV radiation is absorbed. An atmospheric O_2 level of 10^{-1} present atmospheric level (PAL) would have established a significant UV screening ozone shield (Kasting and Donahue, 1980), and an O_2 level of 10^{-2} PAL may have sufficed (Kasting, 1987). There is not a consensus value for the quantity of atmospheric oxygen present during the Archean Eon (3.8 Ga to 2.5 Ga ago). Estimated values range considerably (Rambler and Margulis, 1980; Kasting, 1987). An atmospheric O_2 level of 10^{-5} PAL would be too low to produce ozone and therefore would not attenuate UV radiation. In this situation, the flux reaching the earth's surface in the damaging range of 240 to 270 nm would reach levels of 0.01 to 0.1 $Wm^{-2}nm^{-1}$ (Kasting, 1987). Over this 30 nm range of UV wavelengths, total irradiance might be as high as 1–3 Wm^{-2}. These are speculative values, however, and the UV irradiance might have been considerably lower in the presence of a denser atmosphere and/or other UV absorbing substances.

The emergence of large land plants and animals requires global scale protection from UV radiation which can only be achieved with an atmospheric screen. The emergence of microbial life does not. When considering the size of bacterial cells, an effective screen could be small and irregularly distributed. In addition, protective behavioral responses and internal mechanisms that provide UV protection or repair from UV-induced damage could be utilized to cope with UV radiation. Several theories describing microbial methods for survival in the hypothesized Precambrian environment have been proposed.

The possible screening of microorganisms by liquid water has been suggested (Margulis *et al.*, 1976; Sagan, 1973; Olson, 1981). However, because pure water has a low absorption coefficient for UV radiation, several tens of meters of water would be required to produce an effective shield of UV radiation (Sagan, 1973). Organisms protected by this amount of water could scarcely be considered surface dwelling. Substances dissolved in the water column, such as organic molecules and the inorganic salts, sodium nitrate and sodium nitrite, could provide environmental UV protection. Solutions of these salts absorb strongly in the ultraviolet region. It has been demonstrated that immersion of the cyanobacterium *Lyngbya* in a medium containing high concentrations of these salts results in a mitigation of the lethal effects of UV radiation (Margulis *et al.*, 1976). However, oxidized nitrogenous salts would have been present only in low concentrations in the early Precambrian hydrosphere in the absence of oxygen. Atmospheric screens may have been provided by organic molecules (Sagan, 1973) or elemental sulfur vapor (Kasting *et al.*, 1989). Possible limitations on the atmospheric abundance of organic molecules and the dependence of the sulfur vapor model on other atmospheric constraints (Kasting *et al.*, 1989), reduce the appeal of these suggested solutions.

A likely inorganic UV shield is iron. Ferric iron strongly absorbs in the wavelength range of 220-270 nm. Ferrous iron also absorbs radiation in this range, but less strongly (Olson and Pierson, 1986). Both forms of iron were probably present in Precambrian sediments. The Isua Supracrustals, dating from 3.8 Ga ago, are the oldest known sedimentary rocks and contain banded iron formations in which 30%

of the iron is ferric (Walker *et al.*, 1983). If Precambrian sediments contained more than 0.1% ferric iron, the absorbance at 265 nm of 1.0 mm of sediment would have been substantial (Olson and Pierson, 1986).

Contemporary microbial mats are formed by chemical precipitation and trapping and binding sediment among the mat microorganisms. It can be assumed microbial mats formed 3.5 Ga ago were constructed in similar ways. If the sediments associated with Precambrian microbial mats contained substantial amounts of ferric and/or ferrous iron, they might have protected the microorganisms from UV radiation.

Microbial activities and growth habits also could have contributed to protection from high UV irradiances. Negative phototaxis and photophobic reactions can cause a microorganism to move away from a damaging intense light source. These activities could have protected filamentous gliding bacteria by enabling them to move deeper into the sediments to achieve greater shielding from damaging UV irradiances (Margulis *et al.*, 1976). These types of activities in response to high intensity visible radiation are well-documented in contemporary mat-forming cyanobacteria (Castenholz *et al.*, 1991).

The matting habit is another potentially protective mechanism (Rambler and Margulis, 1980) recently shown to be effective with cyanobacteria (Williamson and Pierson, unpublished observations). In a mat community, cells in the interior are protected from UV exposure by the cells in the surface layers. Following death by exposure, the surface layer of cells continues to provide UV protection to the living cells below until lysis occurs. If the growth and replacement rates of the protected cell layers below are fast enough to exceed the death and lysis rate of the surface-most cells, the population will persist.

Excision repair, photoreactivation, and photoprotection are internal mechanisms used by extant bacteria for protection from and reversal of UV-induced damage to DNA. These mechanisms could have been used by Precambrian microorganisms. Two types of excision repair are known, nucleotide excision repair and base excision repair: Both mechanisms are light independent and utilize internal DNA-editing enzymes (Smith, 1989). Photoreactivation or light-dependent repair is a process by which irradiation of a cell with near UV radiation activates an enzyme, photolyase, which catalyzes the repair of DNA damaged by far UV (UV-C) radiation (Jagger, 1981). It has been suggested (Walter, 1983) that the photoreactivating enzyme may be the oldest of all physiological photoreceptors, and that bacteria may have used light for repair of damaged DNA before they evolved light-dependent energy metabolism.

There are simple and direct protective mechanisms that might have existed in ancient organisms. They might not exist in many of their contemporary descendents due to the lack of recent environmental exposure to high UV fluxes. UV-absorbing pigments could provide a source of protection for cells. Such pigments could be found internally in the cell membrane, cytoplasm or localized in a specialized protective sheath structure external to the membrane and cell wall. Evidence of ensheathed filamentous and coccoid cyanobacteria has been found in the Precam-

brian microfossil record (Schopf and Walter, 1983; Schopf and Packer, 1987). Many contemporary cyanobacteria are known to have sheaths, and many sheaths found on cyanobacteria exposed to high solar irradiances are darkly pigmented (Garcia-Pichel and Castenholz, 1991). The sheath pigment scytonemin absorbs broadly in the near UV (UV-A) (Garcia-Pichel and Castenholz, 1991). Since little UV-C reaches the earth's surface today, there is no need for pigments that absorb broadly through the entire UV range. Pigments absorbing UV-A (from 320–400 nm), such as scytonemin and pigments absorbing UV-B (280–320 nm), such as mycosporines (Karentz *et al.*, 1991) and flavonoids (Cen and Bornman, 1990) provide essential protection. It seems likely, however, that broadly absorbing UV pigments may have been more abundant in sheaths of microorganisms prior to the development of the ozone layer. Other UV-protective pigments may also have been located within the cell and its membranes. Examples of these types of pigments are carotenoids, which appear to provide protection from near UV and visible light damage in most extant organisms growing in high solar irradiances (Paerl *et al.*, 1983; Donkor and Hader, 1991).

Several of the above mentioned external and internal mechanisms for UV protection and/or repair of UV-induced damage may have worked either singly or in various combinations. Evidence for the feasibility of these mechanisms can be obtained by studying extant life forms whose physiological, morphological and ecological characteristics are similar to those of the phototrophs believed to have lived 3.5 Ga ago. Such studies should be conducted under conditions approximating those of the early Earth.

Microorganisms used in previous experiments have rarely been suitable models of Precambrian microbes. Genera which were studied, such as *Bacillus, Escherichia, Clostridium, Sarcina, Pseudomonas* and even the cyanobacteria, *Anacystis* and *Synechocystis*, are not likely representatives of early Precambrian life. These bacteria do not exhibit the characteristcs of Precambrian or even contemporary mat-dwelling microbiota. Additionally, they are all of fairly recent evolutionary origin (Woese, 1987).

Experimental environments have, to date, poorly reconstructed the hypothesized Precambrian environment. Previous experimental approaches were based on subjecting bacteria to brief exposures of UV radiation and plotting the resultant survival curves (Rambler and Margulis, 1980). However, Precambrian microorganisms were exposed simultaneously to radiation in the UV, visible (Vis) and near infrared (NIR) ranges. Addition of visible and infrared radiation to the experimental conditions provides the microorganisms with radiation for photosynthesis and UV-damage repair. Furthermore, most survival curves plotted for organisms exposed to UV radiation have been obtained from organisms exposed to damaging UV radiation under no-growth conditions and usually in the presence of oxygen. It is likely that an organism's response to UV irradiation will be quite different if it is exposed under favorable conditions that support growth and exclude oxygen. Mere survival of UV exposures is not sufficient. Precambrian organisms had to be able to thrive and grow in the presence of high UV fluxes. Therefore, conditions that permit

actual growth of bacteria in environments with high UV irradiances are central to this type of study.

The present study attempts to elucidate the effect UV radiation had on Precambrian microorganisms. We selected an experimental subject which is a good model of Precambrian microorganisms. Subsequently, we assessed the subject's sensitivity to UV radiation under conditions likely to have prevailed in Precambrian environments.

Chloroflexus aurantiacus was chosen for these studies because it is a phototroph from the deepest division in the Eubacterial line of descent containing phototrophic bacteria (Oyaizu *et al.*, 1987). Morphologically, *C. aurantiacus* is a gliding filamentous bacterium similar in appearance to the ancient microfossils of the Warrawoona Group (Schopf and Walter, 1983) and the Onverwacht group (Walsh and Lowe, 1985). *C. aurantiacus* is an extant mat-former (Pierson and Castenholz, 1992). It is therefore more ecologically analogous to the microorganisms which constructed stromatolites than many contemporary bacteria used in previous experiments. *C. aurantiacus* is an anoxygenic phototroph that can be grown and maintained under totally anoxic conditions. Previous studies of the isolation of pigmentation mutants indicate that *C. aurantiacus* has a fair degree of UV resistance (Pierson *et al.*, 1984). In addition, *C. aurantiacus* is a thermophile. This may make it a more suitable candidate as an analog or even a descendent of an early phototroph, if indeed thermophily was a characteristic of the earliest eubacteria (Woese, 1987).

The primary objective of ths study was to determine if *C. aurantiacus* could grow, rather than merely survive, in the presence of UV radiation. The specific questions we addressed were the following:

1. Can *C. aurantiacus* grow in the presence of continuous exposure to UV radiation?
2. Is inhibition of growth in the presence of UV radiation the result of the direct effects of UV irradiation on the cells or the indirect effect of UV-induced alterations in the organic growth medium?
3. What are the effects of increasing UV irradiances on the doubling times and yields of the cultures?
4. What is the maximum UV irradiance in which growth occurs?
5. What is the maximum UV irradiance which is not substantially inhibitory to growth?
6. What is the effect of O_2 on the inhibition of growth by UV radiation?
7. How does the potential for growth of *C. aurantiacus* in the presence of UV radiation compare with that of a known UV-sensitive organism, *E. coli*?
8. Does iron provide an effective shield for high UV irradiances?

Materials and Methods

Organisms

Stock cultures of *C. aurantiacus* strain J-10-fl were grown photoheterotrophically in screw cap tubes in YEDM – medium D containing yeast extract (2.0 g/l) and

glycylglycine (1.0 g/l), (pH 8.2) (Pierson and Castenholz, 1992). Cultures were incubated at 55 °C in incandescent light. Cultures of *E. coli* B (Midwest Culture Service, Terre Haute, Indiana) were grown in T-broth (10.0 g/l tryptone, 5.0 g/l NaCl, pH 6.8) semiaerobically at 37 °C. Experimental cultures were inoculated with exponentially growing stock cultures and adjusted to an initial optical density between 0.02 and 0.04.

Growth of cultures in the presence of UV radiation

Anoxic conditions. Cultures were grown in 250 ml round bottom quartz flasks (Thermal American Fused Quartz Co., Georgetown, DE) containing 65 ml of medium and maintained at 50 °C (*C. aurantiacus*) or 37 °C (*E. coli*) in a water bath. Continuous illumination (320 Wm^{-2}) was provided from a 300 W reflector flood lamp. The flood lamp served as a source of Vis and NIR radiation. UV radiation was delivered continuously from a germicidal lamp (G15T8) (General Electric, Cleveland, OH). The UV irradiance at the surface of the medium was measured using a UV-sensitive radiometer, model IL1350, with a calibrated cosine sensor, SED 40/W (International Light, Newburyport, MA). Greater than 90% of the incident radiation was 254 nm. One quartz culture flask had a hole cut in the bottom permitting insertion of the UV sensor for accurate determinations of the UV irradiance at the surface of the culture before and after each growth experiment. The UV irradiance was increased or decreased by changing the distance between the germicidal lamp and the surface of the culture or by inserting various thicknesses of iron-containing or plain silica gels (see below) between the lamp and the culture. An anoxic atmosphere was created in the culture vessel by continuous sparging with a humidified and filtered mixture of 99.5% N_2 and 0.5% CO_2. The end of the gas dispersion tube was immersed in the medium to achieve mixing as well as dispersion of the gas mixture.

Oxic conditions. Oxic conditions were achieved by growing cultures in 60 ml of medium in a 9×5 cm glass Petri dish covered with a quartz plate. The medium was 1 cm deep and was vigorously stirred with a magnetic stir bar and equilibrated with air. Oxygen concentrations in the cultures during growth were not measured.

Assay Methods. Cultures were grown for a minimum of 48 hours. Approximately every six hours, starting from the time of inoculation, a sample was taken aseptically from the culture vessel using a long-stemmed pasteur pipette and placed in a sterile cuvette. The optical density of the sample at 650 nm was measured with a spectrophotometer (Model 330, Turner Associates, Palo Alto, CA), and the sample was aseptically returned to the culture vessel.

Determination of the effects of irradiated growth medium on the growth of *C. aurantiacus*

Two 70 ml batches of YEDM were prepared for use in these experiments. Both batches were incubated in a quartz culture flask for 24 hours under conditions identical to those used in the UV growth experiments. Both batches of media were

sparged with the nitrogen/carbon dioxide mix, maintained at a temperature of 50 °C, and irradiated with the tungsten reflector flood lamp (300W). Neither batch was inoculated. During the 24-hour incubation period, one batch of medium was continuously irradiated with the UV lamp (0.31 Wm^{-2} at the surface of the medium). The second batch was not exposed to UV radiation. The impact of UV radiation on the ability of the medium to support normal growth was tested by growing cells semi-anaerobically in 7 ml of each medium in screw-cap tubes (10 ml capacity) at 55 °C, 10 cm from two 60 W tungsten lamps. Cultures were inoculated with identical volumes of exponentially growing *C. aurantiacus*. Growth rate and yield were measured by increase in optical density at 650 nm.

Preparation of Silica Gels. Gels of the desired thickness were sandwiched between two circular quartz plates (10 cm in diameter) and mounted between the culture vessel and the UV lamp. Gels were prepared by modification of methods used for the preparation of clear silica gel media for the cultivation of bacteria (Funk and Krulwich, 1964, as described in Krieg and Gerhardt, 1981). Silica gel (16 mesh) was ground to a fine powder, dissolved with heat and stirring in 7% KOH (10g gel in 100 ml KOH), and 20 ml of this solution was mixed well with an equal volume of distilled water. Immediately before pouring the gel, 4 ml of 20% H_3PO_4 were added to the gel solution. The addition of H_3PO_4 lowered the pH of the solution to near neutrality, which was necessary for solidification. Solidification occurred in less than 1 minute. To produce iron-containing gels, the amount of $FeCl_3$ necessary to reach the desired final concentration in the gel was dissolved in the 20% H_3PO_4. Gels with a final $FeCl_3$ concentration of 0.1% or higher contained visible precipitates analogous to precipitated iron found in natural sediments associated with microbial mat ecosystems.

Determination of transmission properties of the gels. The transmission of UV radiation through the gels was measured using the UV radiometer described above. A quartz plate was used to support the gels over the sensor. The germicidal lamp was positioned above the quartz plate so that the incident UV radiation was 2.0 Wm^{-2}. Gels were placed on the plate and the decrease in the transmission of radiation was determined. The transmission of Vis and NIR radiation through the gels was measured as solar spectral irradiance with a spectroradiometer (model LI-1800, LI-COR, Lincoln, Nebraska). The transmission of solar radiation was measured before and after the gels were in place. Spectral irradiance values were integrated over the visible range (400–700 nm) and over the near infrared range (700–1100 nm).

Determination of the transmission properties of the growth media. Absorption spectra of the growth media, YEDM and T-Broth, were determined in the UV and Vis using a Cary 2300 spectrophotometer (Varian Techtron, Mulgrave, Victoria, Australia).

Results and Discussion

Determination of the effects of irradiated growth medium on the growth of *C. aurantiacus*

When growing in a complex organic medium subjected to UV radiation, organisms can be adversely affected in at least two different ways: (1) by the direct action of the radiation on the growing cells and (2) indirectly, by effects of radiation on the medium. Examples of indirect effects are degradation and loss of a required substrate or production of a toxic by-product. We wanted to study the direct effects of UV radiation on *C. aurantiacus*. It was therefore necessary to determine if irradiation of the complex growth medium caused inhibition of growth which could result in misinterpretation of tha data.

Figure 1 illustrates growth curves for *C. aurantiacus* grown in unirradiated and irradiated YEDM. Each curve was based on data from six experiments. The cultures exhibited two different exponential phases of growth. The doubling time for

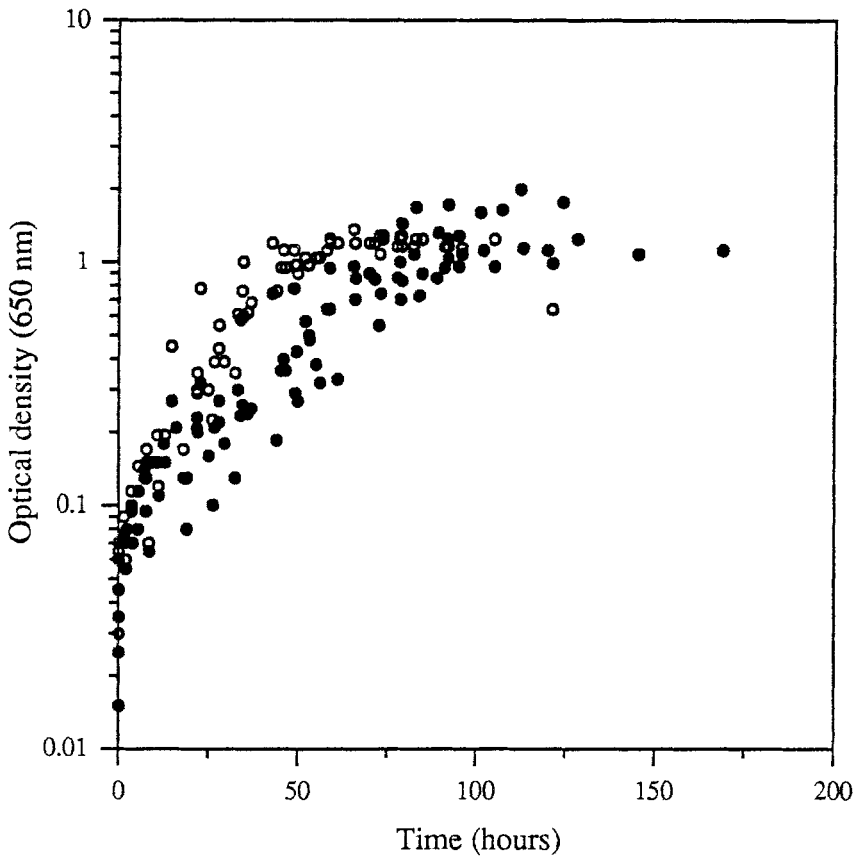

Fig. 1. Growth of *C. aurantiacus* in irradiated (●) and unirradiated (○) organic medium as indicated by the optical density of cultures as a function of time.

the first exponential growth phase of cultures grown in unirradiated YEDM was 3.1 hours, and the doubling time for the second exponential growth phase was 16.5 hours. The yield was 1.30 optical density units. The doubling time for the first exponential growth phase of cultures grown in irradiated YEDM was 10.0 hours, and the doubling time for the second exponential growth phase was 30 hours. The yield was 1.32 optical density units.

It is not unusual for phototrophs growing in a complex medium to exhibit two exponential growth phases. This phenomenon was observed previously in the growth of cultures of *C. aurantiacus*, strain J-10 (Pierson and Castenholz, 1974). The doubling times for cultures grown in irradiated YEDM were longer than the doubling times for cultures grown in unirradiated YEDM. It is possible that UV radiation altered a substrate in the growth medium to a form which was less readily assimilated. Therefore, cultures grown in irradiated YEDM exhibited a depressed growth rate.

Although cultures grown in irradiated YEDM exhibited a depressed growth rate, the ultimate yields at stationary phase for cultures grown in both conditions were identical. Consequently, in subsequent experiments, if the yield of a culture grown in the presence of UV radiation was depressed, we concluded it was due to the direct effect of UV radiation on *C. aurantiacus*. Growth rate could not be used as an indicator of direct UV effects on this organism when grown in the complex organic medium.

The UV absorbance of the organic growth media

C. aurantiacus grows best as a photoheterotroph. Photoautotrophic growth is slow and difficult to maintain with most cultured strains. Although apparently vigorous autotrophic strains of *Chloroflexus*-like organisms have been observed in situ, none has yet been cultured. The growth medium used in these experiments (YEDM) is a rich organic medium with a high UV absorbance that in itself could confer significant UV protection on the cells. Unfortunately this organic medium is not a good analog to Precambrian conditions. Ideally, we would have conducted the experiments with cells grown autotrophically in an inorganic medium. This wasn't possible. Instead we designed a control for the protective effects of an organic growth medium. We grew *E. coli*, an organism known to be UV-sensitive, in an organic medium, in the presence of UV radiation. *E. coli* was grown in T-broth since it could not be grown with near optimal rates in YEDM. Therefore, it was necessary to compare the absorption spectra of the two media to determine if one might confer more protection than the other. The absorption properties of the two media were very siimilar. At 254 nm (the maximum output of the UV lamp), the absorbance of T-broth and YEDM were 7.2 and 7.3 respectively. At 265 nm, the absorbance of T-broth and YEDM were 7.8 and 6.7, respectively. Since the absorbance of YEDM was actually somewhat less than that of T-broth, this organic medium conferred somewhat less protection on *C. aurantiacus* than T-broth did on *E. coli*. The much greater resistance of *C. aurantiacus* to UV damage was therefore due to intrinsic properties of the organism. The protective effects of both media

were minimized by vigorous mixing of the shallow cultures. This prevented the cells from settling to the bottom where they would be shielded by the depth of the medium. All cells should have spent the same amount of time at the top of the culture as at the bottom. When at the top, cells would have been virtually unshielded from the incident UV irradiance.

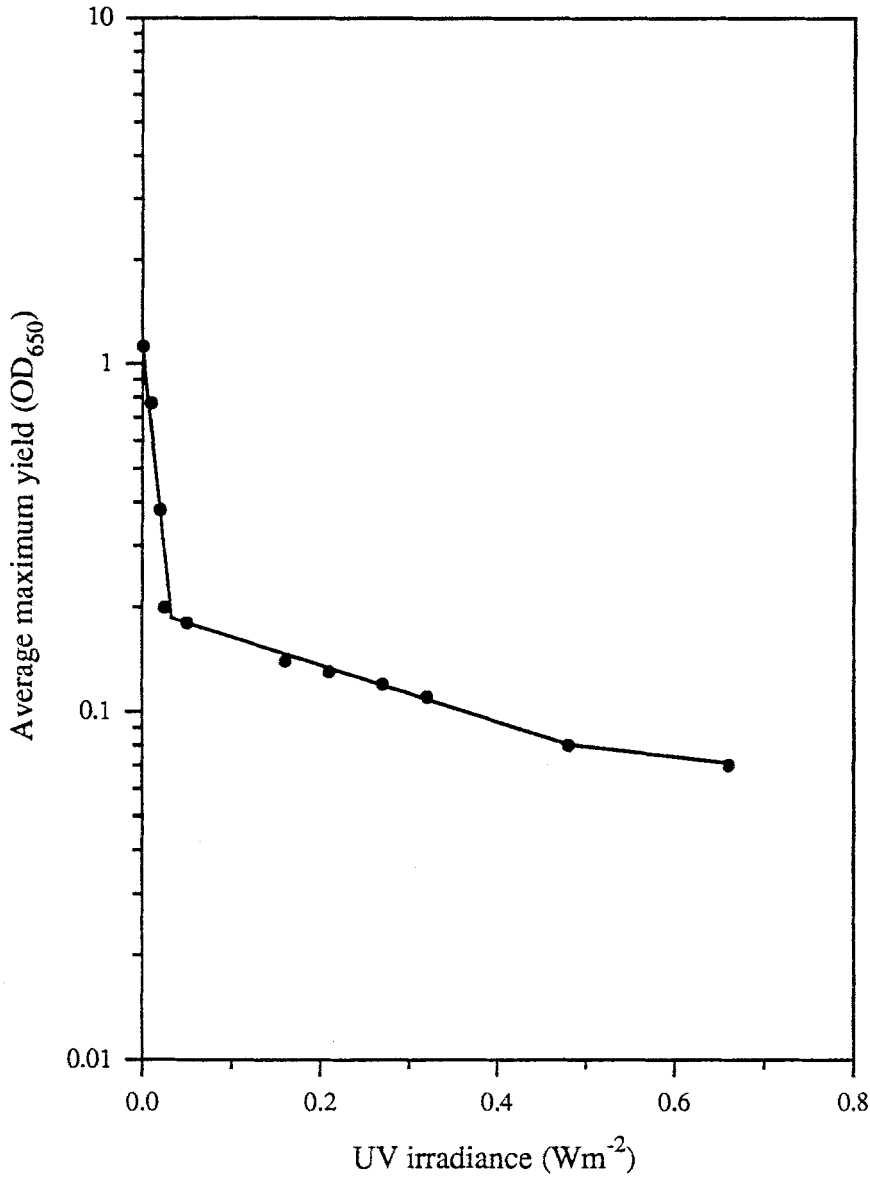

Fig. 2. Semi-logarithmic plot of the yield of cultures of *C. aurantiacus* as a function of the UV irradiance.

TABLE I

Average yield and doubling time for cultures of *Chloroflexus aurantiacus* grown in the presence of UV radiation.

UV irradiance (Wm^{-2})	Average yield (% control) with standard deviation (optical density)	Average T_D (hours)
0.0	1.07 (100) ± .064	3.2
0.01	0.77 (69) ± .064	5.7
0.02	0.38 (34) ± .025	4.2
0.025	0.20 (18) ± .107	3.0
0.05	0.18 (16) ± .071	3.8
0.16	0.14 (13) ± .019	3.3
0.21	0.13 (12) ± .026	4.2
0.27	0.12 (11) ± .043	5.4
0.32	0.11 (10) ± .032	3.9
0.48	0.08 (7) ± .031	6.0
0.66	0.07 (6) ± .025	6.2

GROWTH OF *C. aurantiacus* UNDER ANOXIC CONDITIONS IN THE PRESENCE OF UV RADIATION

Cultures that were grown in the presence of UV radiation and continuously sparged with N_2 and CO_2 exhibited only one phase of exponential growth. Table I lists the average doubling times (T_D) and average yields for the growth of *C. aurantiacus* in the presence of different levels of UV radiation. Generally, as the UV irradiance at the surface of the culture was increased, the yield of the culture decreased. The plot of the yield as a function of the UV irradiance (Figure 2) shows the complexities of the response over a wide range of irradiances. The yield of the cultures decreased exponentially as the UV irradiance increased from zero to 0.05 Wm^{-2}. The rate of decline lessened, and the decrease in yield appeared to be linearly related to the increase in irradiance from about 0.15 to 0.5 Wm^{-2}.

Since the relationship of the doubling time to the UV irradiance was less consistent (Table I) and was complicated by secondary effects of the growth medium (Figure 1), only growth yield was used to assess the effects of UV radiation on these cultures.

Figure 3 plots the average growth curves for *C. aurantiacus* grown at different UV irradiances. Each curve represents 3 or more experiments. The highest UV irradiance used was 0.66 Wm^{-2}. Cultures exposed at this irradiance, and the next lowest value (0.48 Wm^{-2}) exhibited only limited growth (less than 2 doublings). Substantial cell lysis began between 20 and 30 hours after inoculation as indicated by the downward slope of the growth curve from the point of maximum yield. At UV irradiances of 0.32 Wm^{-2} and 0.27 Wm^{-2} measurable growth occurred. Lysis began after 30 hours and was not as extreme nor as rapid as at higher irradiances. Cultures grown in the presence of UV radiation in the range of 0.21 to

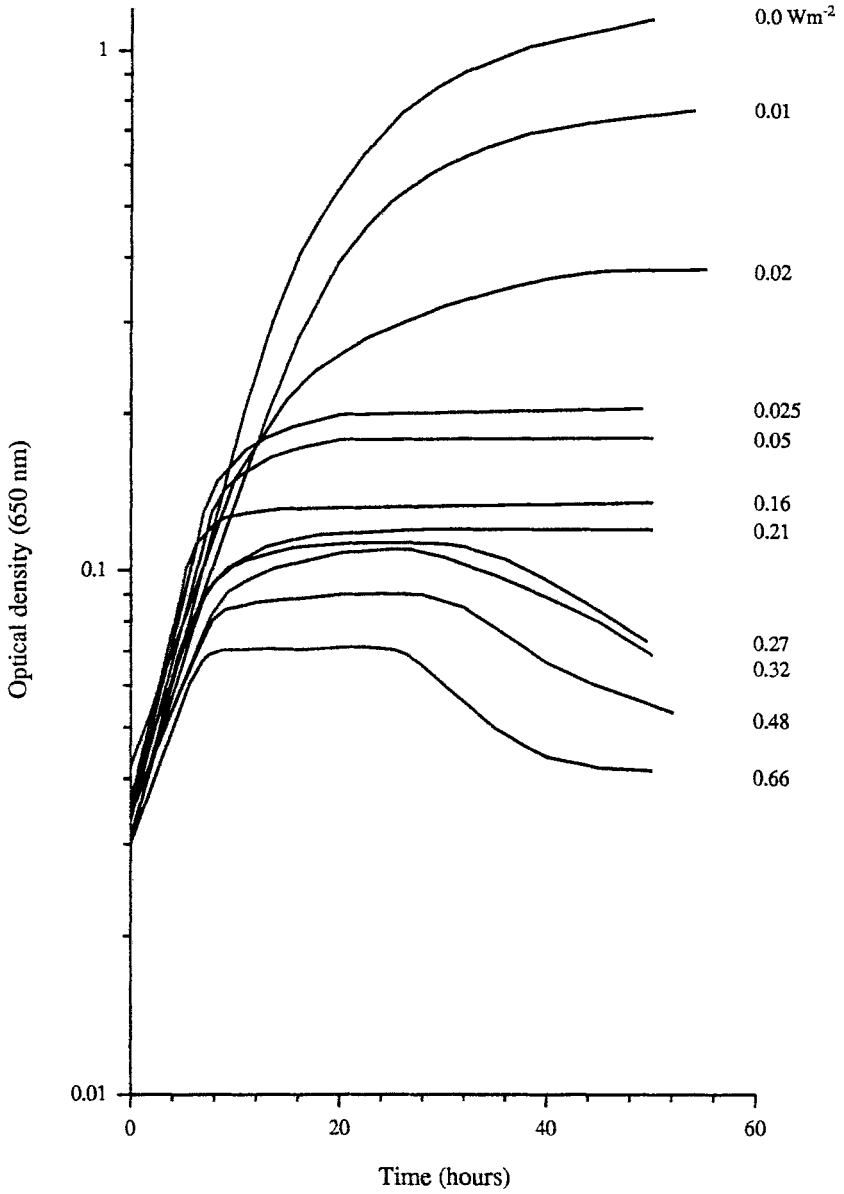

Fig. 3. Growth curves for *C. aurantiacus* grown at different levels of UV radiation. This figure graphically represents the growth curves from which the data in Table I were calculated. Each curve represents best fit by eye from points obtained from 3 different experiments. The approximately 300 data points were omitted for clarity.

0.01 Wm^{-2} did not exhibit lysis. In the presence of the lowest irradiance used, 0.01 Wm^{-2}, the yield was 69% of that of the control.

GROWTH OF *C. aurantiacus* UNDER OXIC CONDITIONS IN THE PRESENCE OF UV RADIATION

C. aurantiacus grows best phototrophically under anoxic conditions but can also grow under oxic conditions as a chemoorganotroph using respiration. If *Chloroflexus*-like organisms evolved early in the Precambrian, it is likely that they were exposed to UV radiation in mats in an anoxic environment. At some point, however, ancestral oxygenic cyanobacteria evolved and began altering the mat environment by making it at least partially oxic at some times of the day. It is possible that the damaging effects of UV irradiation could be enhanced under oxic conditions. Furthermore, our comparative organism, *E. coli*, grew best under oxic conditions and rather poorly under anoxic conditions. Hence, most of our conclusions about the impact of UV radiation on *E. coli* were drawn from experiments done in an oxic environment. In light of these considerations, we decided to evaluate briefly the effects of UV radiation on *Chloroflexus* growing in an oxic environment.

Under oxic conditions in the absence of UV radiation, the doubling time for the growth of *Chloroflexus* was the same as in anoxic conditions. The yield under oxic conditions was much less than under anoxic conditions, however, 0.32 and 1.12 optical density units respectively. The yield under anoxic conditions in the presence of UV radiation (0.02 Wm^{-2}) decreased to 34% of the yield in the un-irradiated control (from 1.12 to 0.38). The yield under oxic conditions decreased to 20% of the control when irradiated (from 0.32 to 0.06). The implication is growth under oxic conditions increases the inhibitory effects of UV radiation. However, we must also bear in mind that these are not optimal growth conditions for this organism. The apparent increased sensitivity of *Chloroflexus* to UV radiation when growing under oxic conditions may reflect intracellular conditions prevailing when growth is suboptimal, rather than any direct potentiation of oxygen for UV damage. Furthermore, the effect of UV radiation on the medium and subsequent growth (Fig. 1) were tested under anoxic conditions. It is possible that UV damage to the medium was different and more toxic under oxic conditions.

THE EFFECT OF UV IRRADIATION OF THE GROWTH OF *E. coli*

E. coli grows best under oxic conditions. In the absence of UV radiation, the cultures grew for 12 to 14 hours before reaching a yield of 0.92. When grown in the presence of an UV irradiance of 0.01 Wm^{-2}, the cultures grew for only 4 hours (3 doublings) before lysis began. The yield was 0.12 (13% of the control). When grown under anoxic conditions in the absense of UV irradiation, the yield was 0.10. When grown in the presence of an UV irradiance of 0.01 Wm^{-2} under anoxic conditions, there were fewer than 2 doublings of the culture and the yield decreased to 0.04 (41% of the control). Lysis occurred after 4 hours. The yield of the irradiated culture was depressed to a much greater degree over that of the unirradiated control, in

TABLE II

Transmission of UV, Vis, and NIR radiation through silica gels with and without iron.

Gel thickness and composition	Transmitted Irradiance (Wm^{-2}) UV (% incident)	Vis (% incident) 400–700 nm	NIR (% incident) 700–1100 nm
Silica gel (no iron)			
quartz plate only	2.00 (100%)	377 (100%)	274 (100%)
1.0 mm gel	1.57 (79%)	337 (89%)	252 (92%)
2.0 mm gel	1.13 (57%)	333 (88%)	252 (92%)
3.0 mm gel	0.78 (39%)	312 (83%)	239 (87%)
Silica gel (0.01% $FeCl_3$)			
quartz plate only	2.00 (100%)	352 (100%)	264 (100%)
1.0 mm gel	0.72 (36%)	306 (87%)	234 (89%)
2.0 mm gel	0.18 (9%)	292 (83%)	230 (87%)
2.5 mm gel	0.105 (5%)	292 (83%)	226 (86%)
Silica gel (0.1% $FeCl_3$)			
quartz plate only	2.00 (100%)	393 (100%)	283 (100%)
1.0 mm gel	0.027 (1%)	335 (85%)	262 (93%)
2.0 mm gel	0.0 (0%)	304 (77%)	252 (89%)
2.5 mm gel	0.0 (0%)	279 (71%)	240 (83%)

the cultures grown in the presence of oxygen than in cultures grown in the absence of oxygen. It appears that oxygen enhances the damage from UV radiation in *E. coli*.

Significantly, under both oxic and anoxic conditions, the yield of *E. coli* was greatly reduced when grown in the presence of an UV irradiance (0.01 Wm^{-2}) that had only a minor impact on the number of generations and subsequent yield obtained with *C. aurantiacus*. A 20-fold increase in irradiance was required to produce similar deleterious effects on *Chloroflexus*.

Iron as an ultraviolet shield

From among the naturally occurring UV shields that might have conferred protection on microorganisms growing in surficial microbial mats during the Precambrian, we chose to test ferric iron. Ferric iron has a very high absorbance throughout the UV and short wavelength visible parts of the spectrum. Therefore, even relatively small amounts of iron present in overlying sediment particles could function as a significant barrier to impinging UV rays. It is not uncommon for mats in shallow aquatic environments to be covered by a few mm of silt or sandy sediments.

Silica gels of the desired thickness were placed on the surface of quartz plate supports, and the transmission of UV radiation through the gels was measured. Various thicknesses of gels containing 0.01% and 0.10% $FeCl_3$ (0.034 and 0.34 ppt Fe^{3+} respectively) were prepared and tested for their transmission of UV radiation. The intensity of the incident UV radiation emitted by the germicidal lamp was

2.00 Wm^{-2} in all cases. The percentage of incident UV radiation transmitted by each gel and the corresponding reduction in irradiance are reported in Table II. Unsupplemented silica gels poorly attenuated UV radiation. The thickest gel tested, 3 mm, transmitted 39% of the incident UV radiation. This irradiance (0.78 Wm^{-2}) would be severely inhibitory to the growth of *C. aurantiacus*. The addition of 0.01% $FeCl_3$ reduced the transmission of UV radiation by a 2 mm gel to 9% of incident. This irradiance (0.18 Wm^{-2}) is a tolerable level for *C. aurantiacus*. A 1 mm gel of 0.1% $FeCl_3$ reduced the transmitted UV radiation to 1% of incident. This irradiance (0.027 Wm^{-2}) is compatible with reasonable growth of *C. aurantiacus*. A 2 mm gel 0.1% $FeCl_3$ completely attenuated the incident UV radiation. Iron supplemented gels proved to be an effective means of regulating the UV irradiance for growth experiments.

The transmission of Vis and NIR radiation through the various thicknesses of unsupplemented and supplemented silica gels was also measured (Table II). A 3 mm gel transmitted 83% and 87% of the incident Vis and NIR radiation, respectively. The addition of iron had a measurable but minor impact on the transmission of photosynthetically active radiation in the Vis and NIR spectra. A 1 mm gel of 0.1% $FeCl_3$, reduced the transmission of incident UV radiation to 1%. The same gel permitted the transmission of 85% and 93% of the incident Vis and NIR radiation, respectively. These experiments suggest levels of particulate iron in the range of 0.034 to 0.34 ppt could be a very effective UV screen for phototrophs growing in shallow or even surficial mat environments. As little as 1 mm of a sediment containing 0.34 ppt Fe^{3+} would suffice and a 3 mm layer of such a sediment would ensure protection. Neither would adversely affect the amount of radiation available in the Vis and NIR needed to sustain photosynthesis.

General conclusions and implications

C. aurantiacus can grow in the presence of continuous UV irradiances that are surprisingly high. Yields and rates for cells grown in anoxic conditions and an UV irradiance of 0.01 Wm^{-2} were similar to control values. This UV irradiance was strongly inhibitory to growth of *E. coli*. It was demonstrated that neither T-broth or YEDM provides significant UV protection. Therefore, we conclude that *C. aurantiacus* has substantial intrinsic resistance to UV radiation. The strain of *C. aurantiacus* used in these experiments lacks a sheath and associated sheath pigments. It is not known whether its ability to grow in the presence of UV radiation is due to intracellular UV-absorbing molecules or to mechanisms for repair of UV damage. As the UV irradiance was increased, growth yields decreased due to the direct effect of UV radiation on the cells. Secondary effects of UV radiation on the growth medium did not affect the yield. Growth was measurable in the presence of a UV irradiance of 0.3 Wm^{-2}, but the yield was less than 10% of that of the control. The fact that growth occurred at all at high UV irradiances was surprising. The difference in reduction of yield at UV irradiances greater than and less than 0.15 Wm^{-2}, suggest the existence of more than one protective mechanism. These

mechanisms may be relics of ancestral mechanisms needed in times of higher environmental UV fluxes. Such mechanisms would be of little use in natural environments today, unless they also conferred protection from damage due to exposure to the near UV part of the spectrum.

C. aurantiacus possesses impressive intrinsic UV-C resistance. We do not know how its degree of UV resistance compares with that of other UV resistant bacteria or with that of other highly pigmented phototrophic bacteria. However, this level of resistance alone would not have been sufficient to protect cells from the high levels of UV-C radiation thought to have prevailed in the Precambrian. Furthermore, the inhibitory effects of UV radiation on *Chloroflexus* appear to be enhanced by oxygen. These two problems prompted us to look for an external mechanism of protection that might have supplemented the resistance conferred by intrinsic protective mechanisms. The combined mechanisms would have to have been sufficient to protect cells under primitive anoxic and early oxic conditions.

We have demonstrated that due to the high UV absorbance properties of ferric iron, low concentrations provide protection. Relatively low levels (0.034 to 0.34 ppt) could be effective shields in sediments 1 to 3 mm thick. Comparable levels of Fe^{3+} and depths of sediment are compatible with the transmission of abundant Vis and NIR radiation to sustain photosynthesis. It has been suggested that photoferrotrophy may have been an early form of photosynthesis (Pierson and Olsen, 1989). Ancestral photosynthetic microorganisms using light energy and ferrous iron as a source of electrons may have constructed their own external UV shields. This would be achieved by deposition of photooxidized iron (Fe^{3+}) waste products outside cells as UV-absorbing ferric salts. The deposits would have accumulated within enveloping sheath or mat-cementing slime. This is similar to the behavior of the extant aerobic ferrous oxidizer, *Thiobacillus ferrooxidans*, which deposits metabolically produced ferric oxides and hydroxides in its immediate environment. Bacterial anionic capsular or sheath material can bind significant amounts of oxidized iron as ferrihydrite and has a much lower affinity for reduced iron (McLean *et al.*, 1992). In the absence of oxygen, photoferrotrophy would have had an advantage as a photosynthetic system. It would have generated an external UV protecting shield and thus permitted organisms to grow at the surface of mat environments where light for photosynthesis was most abundant. The advent of oxygenic photosynthesis might have initially posed a challenge to phototrophs by enhancing the potential for UV damage. However, it would also have contributed to increasing the level of ferric iron in the local environment by chemical oxidation, thereby increasing the density of the external UV screen. In this manner, the exacerbating effects of oxygen on UV damage may have been reduced before the evolution of internal mechanisms to protect cells from photooxidative damage.

We present evidence for the presence of intrinsic UV-resistance mechanisms in the extant mat-forming phototroph, *Chloroflexus aurantiacus*, and evidence for the effective screening of UV radiation by low levels of iron in sediments. This evidence suggests early photosynthetic life could have fluorished in microbial mats in shallow

water environments despite exposure of the Earth's surface to high fluxes of UV radiation. If early atmospheric screens existed and attenuated UV fluxes, organisms such as *C. aurantiacus* with intrinsic UV resistance could have survived. In the absence of such screens, even robust bacteria would have needed the protection of sediment particles and perhaps the mat environment. In either case, it is not likely that UV radiation was a significant deterrent to the early evolution of phototrophic prokaryotes on Earth.

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