Photoreactivation Compensates for UV Damage and Restores Infectivity to Natural Marine Virus Communities†

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We investigated the potential for photoreactivation to restore infectivity to sunlight-damaged natural viral communities in offshore (chlorophyll \(a\), 0.1 \(\mu g\) liter\(^{-1}\)), coastal (chlorophyll \(a\), 0.2 \(\mu g\) liter\(^{-1}\)), and estuarine (chlorophyll \(a\), ca. 1 to 5 \(\mu g\) liter\(^{-1}\)) waters of the Gulf of Mexico. In 67% of samples, the light-dependent repair mechanisms of the bacterium Vibrio natriegens restored infectivity to natural viral communities which could not be repaired by light-independent mechanisms. Similarly, exposure of sunlight-damaged natural viral communities to >312-nm-wavelength sunlight in the presence of the natural bacterial communities restored infectivity to 21 to 26% of sunlight-damaged viruses in oceanic waters and 41 to 52% of the damaged viruses in coastal and estuarine waters. Wavelengths between 370 and 550 nm were responsible for restoring infectivity to the damaged viruses. These results indicate that light-dependent repair, probably photoreactivation, compensated for a large fraction of sunlight-induced DNA damage in natural viral communities and is potentially essential for the maintenance of high concentrations of viruses in surface waters.

The UV-B radiation (280 to 320 nm) that reaches the Earth’s surface can penetrate to considerable depth in seawater (27) and cause significant damage to a variety of marine microorganisms (1, 4, 11, 13, 15, 23, 38), including viruses (24, 29, 32). While UV-B radiation is thought to be the component of sunlight which inhibits biological activities, longer wavelengths (UV-A [320 to 400 nm] and photosynthetically active radiation [400 to 700 nm]) may also cause damage (3, 26, 32). A major target of UV radiation is cellular DNA, although other molecules (i.e., protein) may also be affected. Given that the infectivity of viruses is extremely sensitive to solar radiation (24, 29, 30, 32), and that damage to viral infectivity is proportional to the radiation received (24, 25, 29), it is puzzling that viruses remain extremely abundant in marine surface waters. Indeed, studies have suggested that the viral production rates that would be necessary to balance measured decay rates of infectivity are, in some cases, unsustainable (29, 30, 32). Potentially, induction of lysogenic viruses by sunlight could counterbalance losses of infectivity; however, several studies have indicated that induction of lysogens is unlikely to be a major source of viral production (14, 36, 37). Another explanation to the paradox of high infectious virus concentrations in the presence of high doses of UV radiation is that the damage to the viral DNA is repaired. The most important types of UV-B-induced DNA damage are cyclobutane pyrimidine dimers and pyrimidine-6-4 pyrimidone photoproducts (19). The formation of these photoproducts in cellular DNA can result in reduced cell viability and even death (8). DNA damage in bacterial and algal cells can be repaired by light-dependent (photoreactivation) as well as light-independent (dark repair) mechanisms. While some viruses encode for their own DNA repair enzymes (17, 18), most viruses rely on the DNA repair mechanisms of host cells (2). While the loss of infectivity associated with exposure to UV radiation is typically caused by damage to the viral DNA, repair is possible only after the DNA has been injected into the host cell.

The process of photoreactivation was originally described as the restoration of infectivity to UV-irradiated viruses upon exposure to visible light in the presence of host bacteria (5, 6). It has since been demonstrated that the blue light (300 to 500 nm)-activated enzyme photolyase reverses the dimmerizing effects of UV-B in the damaged DNA of cells as well as in infecting phages (8, 16). Recently, the crystal structure of the photolyase of Escherichia coli has been determined (20), and photolyases that can remove another type of DNA damage, the pyrimidine-6-4 pyrimidone photoproduct, were found (33). Examples of photoreactivation occur in all three domains of life, although it is absent in many species (8). Most work on photoreactivation has been performed with members of the domains Archaea and Bacteria. For example, in the archaeon Halobacterium cutirubrum, photoreactivation is very efficient in removing cyclobutane dimers, whereas dark repair is apparently absent (7). Photoreactivation has also been studied extensively in bacteriophages (see, e.g., references 2, 5, 6, 22, and 35); however, in most of these studies DNA was damaged with monochromatic light (typically 254 nm) and photolyase was induced with white light. Although findings indicate that photoreactivation is a major mechanism responsible for the repair of UV-induced DNA damage, studies have not examined the role of light-dependent repair in restoring infectivity to natural viral communities which have been damaged by sunlight.

Since photoreactivation could be the mechanism allowing for the persistence of viruses in surface waters, we investigated the potential for light-dependent repair mechanisms to restore infectivity to natural populations of phages which infect Vibrio natriegens, as well as to entire viral communities from coastal and offshore waters of the Gulf of Mexico. We found that light-dependent repair mechanisms restored infectivity to a
significant portion of naturally occurring viruses which could not be repaired by light-independent mechanisms. Our results indicate that in many locations, photoreactivation is probably necessary for maintaining high concentrations of infectious viruses in marine surface waters.

MATERIALS AND METHODS

Study sites and sampling. Seawater for experiments was collected from the pier of the Marine Science Institute of the University of Texas at Austin (Port Aransas, Tex.), as well as from several stations in the Gulf of Mexico (Fig. 1), by pumping water from a depth of ca. 0.5 m or by using Go-Flo bottles mounted on a rosette. Duplicate subsamples for the enumeration of viruses and bacteria, and for the determination of burst size, were preserved with glutaraldehyde (2%).

Experiments with natural viral populations infecting V. natriegens. Vibrio natriegens strain PWH3a (strain PWH3a) originally isolated from the Gulf of Mexico (32) was used as the host to determine the abundance of viruses in natural waters in which infectivity could be restored via light-dependent repair in this bacterium. Large volumes of seawater (100 to 200 liters) were collected between 1000 and 1800 h, from depths between 0 and 15 m. The samples were filtered (1.2-μm-nominal-pore-size glass fiber filter [Gelman GC50] and 0.2-μm-pore-size polycarbonate cartridge filter [Nuclepore]) to remove zooplankton, phytoplankton, and bacteria. The viruses remained in the 0.2-μm filtrate and were concentrated to a final volume of 100 to 200 ml by ultrafiltration (30,000-molecular-weight cutoff; Amicon S10Y30 and S1X30 cartridges) as described in Suttle et al. (31). The light-dependent restoration of viral infectivity for these natural populations of phages was determined by plaque assay (32) with V. natriegens under photoreactivating wavelengths and nonphotoreactivating (dark) conditions. Photoreactivating conditions were brought about by exposure of the virus-host cell mixture for 30 min to 200 μmol m⁻² s⁻¹ of photosynthetically active radiation with wavelengths of <312 nm removed (Mylar D-screened Philips F40CW Cool White fluorescent tubes) prior to plating. Maximum titers of infective viruses that had been exposed to sunlight occurred after treatment with photoreactivating conditions for longer than 15 min.

Experiments with natural viral communities. The following approach was used to examine the role of host-mediated light-dependent repair in restoring infectivity to entire natural viral communities which had been exposed to damaging levels of solar radiation. For each experiment a seawater sample (4 liters) was collected before sunrise and filtered through glass fiber and cartridge filters as described above to remove zooplankton, phytoplankton, and bacterioplankton. Aliquots (250 ml) of the filtrate containing the natural viral community were dispensed into polyethylene bags and exposed to sunlight during cloudless days from ca. 0800 to 1030 h or were kept in the dark. The sunlight-exposed and unexposed viruses were then added back to aliquots of the bacterial community which had also been harvested before sunrise and concentrated about 10-fold by using the 0.2-μm-pore-size cartridge filter. The addition of the viruses diluted the bacteria to approximately ambient concentrations. This resulted in abundances of viruses and bacteria, and hence in encounter rates between viruses and host cells, that were similar to those in the original seawater samples. The samples were placed in polyethylene bags and incubated under photoreactivating wavelengths from ca. 1100 to 1800 h or were maintained in the dark to prevent photoreactivation. Sunlight was attenuated to 30% by neutral density screening, and Mylar D was used to exclude wavelengths of <312 nm to prevent further DNA damage during the photoreactivation period. These treatments minimized DNA damage to the bacteria and viruses but allowed exposure to photoreactivating wavelengths (300 to 500 nm) (8, 37a). Following exposure to photoreactivating light, the bags were transferred to the dark. All treatments, including dark controls, were duplicated and kept at in situ temperatures with flowing seawater.

To check for potential toxic effects of polyethylene bags, viruses were exposed to solar radiation and incubated with the bacterial community under photoreactivating light in glass petri dishes in parallel with the polyethylene bags. As well, viruses in water collected from the pier of the Marine Science Institute were exposed to solar radiation and incubated along with the natural bacterial community under wavelengths of >550 nm (Acrylite 436-4; Cryo Industries), >370 nm (Plexiglas), or >312 nm (Mylar D) or in the dark in order to identify the wavelengths responsible for light-dependent repair and to determine if effects other than photoreactivation might be occurring. Samples were removed periodically from the incubations and preserved in glutaraldehyde (2%).

Bacterial and viral counts. Glutaraldehyde-processed samples were kept at 4°C in the dark and processed within 2 weeks. Bacteria and viruses were stained with 4',6-diamidino-2-phenyindole (DAPI; final concentration, 1 μg ml⁻¹), filtered onto 0.02-μm-pore-size Anodisc filters (Whatman), and enumerated by epifluorescence microscopy (Olympus BX40 microscope; excitation, <400 nm; dichroic filter, 500 nm; barrier filter, >515 nm) by modifying the protocols of Turley (34) and Suttle (29) as described in Weitzel and Suttle (36). In order to confirm that DAPI counts of virus particles were accurate, estimates of viral abundance were also made by staining with Yo-Pro-1 [4-[3-methyl-2,3-dihydroxy-(benzo-1,3-oxazole)-2-methylthiophenolide]-1,3-(trithymallymoniumumpropyl)]-quinolinium diiodide] (10); results with the two methods were not significantly different (data not shown; P > 0.05 [n = 7] in a two-tailed paired t test). As well, bacteria were counted on 0.2-μm-pore-size Anodisc filters (Whatman), and abundances were not significantly different from those obtained on 0.02-μm-pore-size filters that were prepared for counting viruses (data not shown; P > 0.5 [n = 7] in a two-tailed paired t test). Therefore, bacteria and viruses were counted on the same 0.02-μm-pore-size filters. Chlorophyll a concentrations were determined fluorometrically (21) on 250- to 1,000-ml samples filtered onto 0.2-μm-nominal-pore-size nitrocellulose filters.

Calculation of light-dependent repair. The restoration of viral infectivity by light-dependent repair was inferred by comparing changes in viral abundance among samples in which sunlight-exposed viruses were incubated along with the natural bacterial community either under photoreactivating wavelengths or in the dark. The assumption made in these experiments is that the number of viruses produced is proportional to the number of infectious viruses at the beginning of the incubation. First, the loss of viral production as a result of sunlight-induced damage was calculated from the difference between the number of viruses produced (V) in treatments which were exposed (e) or not exposed (u) to sunlight (37a).

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V = (u - e) / e 
\]

where V is the relative loss of viral production as a result of sunlight exposure (37a). A negative value of V indicates that viral production is increased by exposure to sunlight. A positive value of V indicates that viral production is decreased by exposure to sunlight. A value of V close to zero indicates that viral production is not affected by exposure to sunlight. The loss of viral production as a result of sunlight-induced damage was calculated from the difference between the number of viruses produced (V) in treatments which were exposed (e) or not exposed (u) to sunlight (37a).

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to UV radiation, and then incubated along with the natural bacterial community in the dark (d) or under photoreactivating light (p). Light-dependent repair (LDR) was expressed as the percentage of viruses damaged by sunlight in which infectivity could be restored under photoreactivating conditions: %LDR = 100 \times \frac{(V_{pe} - V_{de})}{(V_{du} - V_{de})}

### RESULTS AND DISCUSSION

**Station characteristics.** Based on chlorophyll a concentrations and location, the stations could be grouped into oligotrophic and oceanic waters (<0.1 \mu g liter\(^{-1}\); stations B and C), moderately productive shelf waters (ca. 0.2 \mu g liter\(^{-1}\); stations E and F), and highly productive nearshore waters (ca. 1 to 5 \mu g liter\(^{-1}\); pier) (Table 1). The penetration of UV and the mixing depth were determined only for the oceanic and shelf stations. The 1% light penetration for UV-A was much deeper than for UV-B at the oceanic stations (ca. 123 versus 24 m) and the shelf stations (ca. 10 versus 2 m). The mixing depth ranged from 22.3 to 28.7 m at stations B, C, and D and was 10.2 m at station E.

**Light-dependent repair of viruses infecting *V. natriegens*.** Light-dependent repair of infectivity was estimated in viral communities collected between 1000 h and 1800 h from several depths, corresponding to UV-B irradiances ranging from 1 to 100% of the surface (Table 1). In 67% (n = 21) of the samples, the abundance of infective viruses increased relative to that of dark controls when the host cells (*V. natriegens*) were exposed to photoreactivating wavelengths (Table 2). The concentration of viruses infecting *V. natriegens* increased by 1.1 to 8.5 times (mean, 3.0; standard deviation, 2.17) in samples exposed to photoreactivating light relative to those in dark controls. Light-dependent repair was not detected in viral communities that were collected before sunrise or from depths below the penetration of UV-B light (data not shown). As the abundance of viruses infecting *V. natriegens* increased under photoreactivating conditions relative to dark controls, it appears that light-dependent repair restored infectivity to viruses that could not be repaired by light-independent (dark) mechanisms. As the light-activated enzyme photolyase will repair DNA damage caused by sunlight (8), this enzyme was most likely responsible for restoring infectivity in these experiments.

### Table 2. Restoration of infectivity to viruses infecting *V. natriegens* and to natural viral communities by photoreactivating wavelengths

<table>
<thead>
<tr>
<th>Station and mo</th>
<th>No. of samples in which viruses infecting <em>V. natriegens</em> were repaired/total</th>
<th>% of damaged viruses in natural viral communities in which infectivity was restored</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>3/6</td>
<td>26.3</td>
</tr>
<tr>
<td>C</td>
<td>3/6</td>
<td>21.4</td>
</tr>
<tr>
<td>E</td>
<td>3/3</td>
<td>51.8</td>
</tr>
<tr>
<td>F</td>
<td>2/2</td>
<td>41.4</td>
</tr>
<tr>
<td>Pier</td>
<td></td>
<td></td>
</tr>
<tr>
<td>June</td>
<td>ND</td>
<td>48.5</td>
</tr>
<tr>
<td>July</td>
<td>3/4</td>
<td>42.8</td>
</tr>
<tr>
<td>Oct.</td>
<td>ND</td>
<td>41.2</td>
</tr>
</tbody>
</table>

*For details of sample locations, see Table 1, footnote a.

**FIG. 2.** (A) Hypothetical production of viruses in treatments to demonstrate how experimental data on viral and bacterial production were interpreted. The results for both dark treatments would be expected to be similar, hence, the data overlie one another. (B and C) Sample data set showing viral and bacterial production, respectively, in experiments performed in June 1995 with water collected at the pier of the Marine Science Institute. Data are presented as means (± ranges) of duplicate incubations. Where error bars are not shown, differences between duplicates were not detected. PR, photoreactive.
Light-dependent repair of entire natural viral communities. Changes in viral abundance showed a similar pattern in all experiments (Fig. 2 and 3). Viral production was lowest when viruses which had been damaged by sunlight were incubated in the dark along with the natural bacterial communities, while production was greatest when viruses which had not been exposed to sunlight were incubated with bacterial communities in the dark or under photoreactivating wavelengths. Viral production was reduced up to 68% (mean, 45% ± 24.5%) in dark incubations to which sunlight-damaged viruses were added relative to incubations to which undamaged viruses were added. However, when sunlight-damaged viruses were added to natural bacterial communities under photoreactivating wavelengths (>312 nm), the number of viruses produced was consistently greater than when sunlight-damaged viruses were added to natural bacterial communities and incubated in the dark.

These results demonstrate that the infectivity of viruses which could not be repaired by natural bacterial communities in the dark could be repaired by the same bacterial communities under photoreactivating wavelengths. Viral production was not significantly different between dark and photoreactivating-light incubations to which undamaged viruses were added. Therefore, photoreactivating light did not affect viral production other than by inducing light-dependent repair mechanisms.

The potential of photoreactivating wavelengths to restore infectivity was examined for each sample by comparing viral production in sunlight-damaged samples incubated under photoreactivating and nonphotoreactivating wavelengths (Fig. 3). In samples from the transparent waters of the central Gulf of Mexico (stations B and C), only about 21 to 26% of the damaged viruses were repaired by light-dependent mechanisms (Table 2), whereas in samples from the more turbid stations (stations E and F, and the pier), where damage to the viruses may have been less, 41 to 52% of the damaged viruses were repaired. Overall, differences in viral abundances between incubations that received sunlight-damaged viruses which were kept under photoreactivating light and those kept in the dark were significant (P < 0.01 in one-tailed paired t tests comparing viral production under photoreactivating and nonphotoreactivating conditions at each station). These data clearly demonstrate that light-dependent repair can restore infectivity to a significant proportion of viruses in seawater that have been damaged by solar radiation.

Polyethylene bags exposed to UV-B have been reported to produce a toxin that lowers photosynthetic rates in phytoplankton (12); therefore, we performed an experiment with a natural viral community in which samples were incubated in polyethylene bags and glass petri dishes in parallel and exposed to full sunlight and photoreactivating light. Since viral and bacterial production levels were not different between the polyethylene-bag and glass-petri-dish incubations (Fig. 4), it is unlikely that the polyethylene bags affected viral and bacterial production in our experiments.

Additional experiments were conducted in order to confirm that photoreactivation was the process likely responsible for the observed light-dependent repair. Our original experiments used neutral density screening and Mylar D to attenuate sunlight and exclude damaging wavelengths of <312 nm but still allow for photoreactivation. However, wavelengths of >312 nm could potentially damage host cell DNA and induce SOS repair (a light-independent mechanism), which would be confused with light-dependent repair in these experiments. Therefore, we used Plexiglas, which excludes damaging wavelengths (<370 nm) but allows for photoreactivation, and Acrylite, which excludes wavelengths of <550 nm and prevents photoreactivation. Viral production levels in all treatments with Plexiglas and Mylar D were not different from each other but were higher than levels in the Acrylite and dark treatments, which were also similar to each other (Fig. 5). The data demonstrate that the wavelengths responsible for restoring viral infectivity were between 370 and 550 nm, which includes the absorbance peaks of the two known classes of photolyases (ca. 380 and 440 nm) (8). The data also indicate that Mylar D excluded wavelengths which could damage the host cells and induce other DNA repair systems. Moreover, viral and bacterial production levels were not significantly different between incubations to which undamaged viruses were added which were kept in the dark and those exposed to photoreactivating light (Fig. 2 and 3). Therefore, photoreactivating light did not affect bacterial or viral production other than by inducing light-dependent repair mechanisms.

Photoreactivation is currently defined as the removal of cyclobutane pyrimidine dimers by photolyase activity (8). Measurements in the Gulf of Mexico have demonstrated that there is a positive correlation between the formation of pyrimidine dimers in virus DNA and the loss of viral infectivity (35a). However, it is not possible to directly measure photolyase activity by the removal of pyrimidine dimers because every
virus that is repaired will replicate and produce a large but unknown number (usually 10 to 100) of progeny viruses, thereby diluting the concentration of photoproducts. This is compounded by replication of undamaged viruses. As a consequence, the removal of photoproducts by photoreactivation cannot be separated from dilution due to viral production.

Our data cannot be used to quantify dark repair. However, in an experiment performed at station E, there was no measurable viral production in the dark incubations containing damaged viruses, whereas there was a significant increase in viral abundance in identical samples which were incubated under photoreactivating light (Fig. 3). This indicates that photoreactivation was much more efficient than dark repair in restoring infectivity to viruses damaged by UV radiation. It is possible that there was too little damage to the phage DNA to induce the host dark-repair system. This is supported by the finding that the infectivity of UV-damaged λ phage is restored at a higher rate when E. coli cells are irradiated with UV to induce the SOS repair response prior to infection than when host cells are not irradiated (8). Photoreactivation uses photons and an enzyme to directly reverse DNA damage and is energetically less costly than dark repair, which requires the formation of new nucleotides and the combined action of several enzymes. Thus, in environments in which DNA damage is caused by solar radiation and nutrients are in short supply, it should be advantageous for bacteria to use photoreactivation to repair DNA damage, given that sunlight is plentiful.

Filtration and concentration decreased viral and bacterial abundances to 56.7 to 100.5% (mean, 87.7% ± 17.3%) and 42.8 to 105.4% (mean, 78.7% ± 26.5%), respectively. Therefore, encounter rates between viruses and bacteria in the experiments were only slightly lower than those in nature. During experiments bacterial numbers increased by about two- to threefold and did not differ greatly among treatments (see, e.g., Fig. 2). It might be expected that bacterial abundances would be highest in treatments with the lowest viral production (i.e., samples with damaged viruses incubated with bacteria in the dark). However, lysis products of bacteria potentially stimulate bacterial production (9), which would confound interpretation of the results. Increases in bacterial abundance would also be expected because of the removal of grazers during prefiltration and the removal of UV radiation during the incubations. Similarly, the removal of grazers and reduction in UV radiation may have influenced viral abundance, but these effects would have occurred in each treatment and thus would not have affected the interpretation of the results.

The data presented here indicate that the loss of viral infectivity resulting from solar radiation may be largely offset by host-mediated light-dependent repair of the damaged DNA. The extent to which light-dependent repair can restore viral infectivity will depend on the transparency of the water column and the depth of mixing. The deeper the mixing depth relative to the penetration of UV-B, the less damage will occur. At stations B and C, the depths of mixing and 1% light penetration of 305-nm-wavelength light were approximately the same, whereas at stations E and F, the light penetration depth for 305-nm-wavelength light was less than ca. 20% of the mixing depth (Table 1). As photoreactivating wavelengths penetrate deeper than DNA-damaging radiation (e.g., 380-nm radiation penetrated four to six times deeper than 305-nm radiation [Table 1]), the mixing of viruses below a critical depth should allow light-dependent repair to occur in the absence of further damage.

The ability of photoreactivating enzymes to restore infectivity to viruses exposed to UV radiation is well known (5, 6, 8); however, the significance of this process to viral populations in nature has never been established. Our results clearly demonstrate that the photoreactivating mechanisms of natural marine bacteria communities are important and may be essential for maintaining high concentrations of infectious viruses in the sea. These data provide an explanation for the apparent paradox of high concentrations of infectious viruses in the sea in the presence of high doses of solar radiation (29, 30, 32).

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