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Assessment of UV-B damage in cyanophage PP

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ABSTRACT: Cyanophage PP is a short-tailed, icosahedral-shaped, double-stranded DNA virus and can be frequently detected with high abundance and activity in many eutrophic lakes in China. Solar radiation is one of the main factors affecting cyanophage infectivity. In this study, cyanophage PP was treated with different intensities of UV-B radiation, and the accumulation of cyclobutane pyrimidine dimers (CPDs), viral particle destruction and viral attachment ability were analyzed. The viral infectivity decay rate under white and red light was also studied using plaque-forming assays. The results indicate that (1) the level of CPDs induced by UV-B was significantly correlated with radiation intensity, while exposure dose had little effect on the DNA damage level, (2) the level of CPDs was significantly correlated with both the decay rate and the repair rate in cyanophage PP, and (3) UV-B could result in the destruction of viral particles and the decrease in viral attachment. This work suggests that the secondary and tertiary structure of the DNA may influence the formation of CPDs, and that capsid damage caused by UV-B radiation may play an important role in viral decay and survival.

KEY WORDS: Cyanophage PP · UV-B · Cyclobutane pyrimidine dimers · CPDs · Capsid damage

INTRODUCTION

Aquatic viruses, including cyanophages, play key roles in microbial food webs in aquatic ecosystems (Fuhrman 1999, Tarutani et al. 2000, Suttle 2007). Damage caused by solar ultraviolet radiation (UVR) is one of the main factors affecting viral infectivity in seawater (Noble & Fuhrman 1997, Garza & Suttle 1998). Virus inactivation rates are about 10 times higher in sunlight than in the dark (Suttle & Chen 1992, Sinton et al. 2002).

The UV-B component of solar radiation is directly absorbed by viral DNA, resulting in the formation of cyclobutane pyrimidine dimers (CPDs) (Weinbauer et al. 1999, Hader & Sinha 2005). It is widely accepted that these photoproducts prevent the replication of DNA, hence affecting virus infectivity. UV-B may also result in the formation of reactive oxygen species (ROS) including singlet oxygen ($^1O_2$) or free radicals, which can destroy the structure of biological molecules such as membrane proteins and lipids (Alscher et al. 1997, McKenzie et al. 2003). UVR-induced capsid damage has been reported for several pathogenic RNA viruses that are released into aquatic systems through drainage systems (De Sena & Jarvis 1981, Wetz et al. 1983, Nuanualsuwan & Cliver 2003). UV irradiation of type I poliovirus resulted in modification of the poliovirus capsid and loss of infectivity (De Sena & Jarvis 1981, Wetz et al. 1983). The primary target of UVR and other virus inactivation factors is the capsid (Nuanualsuwan & Cliver 2003). Strong sunlight affects the viability of viral particles in surface waters and decreases with depth in the marine water column (Wommack et al. 1996, Wilhelm et al. 1998). These results imply that UVR-induced capsid damage may also be an important factor in the viral inactivation process.

UV damage to aquatic viruses is affected by many factors. Studies of marine bacteriophage indicate that season, water depth and exposure dose affect the decay rate of the virus (Wommack et al. 1996, Garza & Suttle 1998, Wilhelm et al. 1998). Examinations of latitudinal gradients have also shown that the level of viral DNA damage is correlated with the irradiation density, but these parameters are not necessarily linear and

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other factors (e.g. water column chemistry and composition, virus community diversity) may also be involved (Wilhelm et al. 2003).

Cyanophage PP was isolated from a lake in China and can infect 2 hosts, *Plectonema boryanum* and *Phormidium foveolarum*. It is a short-tailed, icosahedral-shaped, double-stranded DNA virus, 52 nm in size and with a genome of 30 kbp (Zhao et al. 2002). This cyanophage is found in high abundance and with high activity in many eutrophic lakes in China (Zhao et al. 2002, Cheng et al. 2007). In our previous work, we investigated solar UVR damage and photoreactivation of cyanophage PP in a shallow freshwater lake. Our investigation demonstrated that rapid infectivity decay of the cyanophage induced by solar UVR can be compensated for by rapid photoreactivation by host cyanobacteria. Furthermore, our previous results indicated that the cyanophage decay rate quickly reached saturation, suggesting that the effects of UVR were rapidly balanced by resistance mechanisms (Cheng et al. 2007). In this study, we further investigated the mechanism of UV-B damage in cyanophages by analyzing the accumulation of CPDs in the cyanophage PP genome, viral capsid integrity and the ability of viruses to attach under different UV-B intensities. The results indicate that the secondary and tertiary structure of the DNA may influence the formation of CPDs, and that capsid damage caused by UV-B radiation may play an important role in viral decay and survival.

MATERIALS AND METHODS

Cyanobacteria and cyanophages. The cyanobacterial strain, the axenic *Plectonema boryanum* IU594, originally obtained from the Freshwater Algae Collection of the Institute of Hydrobiology, Chinese Academy of Sciences, was cultured in Allen and Arnon (AA) medium at 28°C (Allen & Arnon 1955, Cheng et al. 2007). White light (240 µE m^{-2} s^{-1}) was provided by white fluorescent tubes (Osram L 18W/160) and the photocycle was set to 16 h light:8 h dark.

Cyanophage PP was prepared as follows: 10 l of *Plectonema boryanum* IU594 culture (10^8 cells ml^{-1}) was inoculated with 100 ml of 10^8 plaque-forming units (PFU) ml^{-1} of cyanophage PP. After incubation at 28°C for 24 h, the cyanobacteria were completely lysed. The lysed culture was centrifuged under 10 000 × *g* for 10 min at 4°C, and the supernatant was digested with DNase I (0.1 mg ml^{-1}) and RNase (0.1 mg ml^{-1}) at room temperature for 1 h. The digested supernatant containing cyanophage PP was ultrafiltrated through a tangential flow filtration system with an ultrafiltration membrane (Pall corporation, 100 kDa molecular weight cutoff) (Luo et al. 2003). The cyanophage PP was ultrafiltrated and condensed to 400 ml and stored at 4°C in chloroform (10%) (Luo et al. 2003).

Sample treatment. Three Petri dishes each containing 100 ml of the condensed cyanophage PP were exposed to a UV-B lamp (270 to 400 nm wavelength) (BLE-1T158, Spectroline) 30 cm above the dishes. Two of the dishes were covered with different filters to produce radiation intensities of 2.5 and 14 µW cm^{-2}, respectively. The radiation intensity delivered to the Petri dish without a filter was equivalent to 60 µW cm^{-2}. These radiation intensities corresponded to those at 20 cm below the surface of the water in Donghu Lake in Wuhan (a city in the center of China), the surface of the water in Wuhan in summer, and the surface of the water in Kunming (a city in southern west of China), respectively. Ten ml of each sample was taken at different times post radiation, and a final dose of 1080 J m^{-2} irradiation was provided to each treatment. Unirradiated cyanophage was used as a control.

Enzyme linked immunosorbent assay (ELISA) test for UVR damage. Eight ml of each treated sample was ultrafiltrated and washed with phosphate-buffered saline (PBS) (137 mmol l^{-1} NaCl, 2.7 mmol l^{-1} KCl, 10 mmol l^{-1} NaH_2PO_4, 2 mmol l^{-1} KH_2PO_4, pH 7.5) in ultrafilter tubes (30 000 MWCO, Millipore) and concentrated to 0.5 ml. Viral DNA was extracted as described by Sambrook et al. (1989) and dissolved in PBS at a concentration of 0.2 µg ml^{-1}. ELISA was carried out to examine the changes in CPDs. Briefly, 50 µl of each denatured DNA solution was added to protamine sulfate-precoated 96-well microtiter plates and dried overnight at 37°C. Then, 150 µl of 2% fetal bovine serum in PBS was added for 30 min to prevent nonspecific antibody binding. Next, 100 µl of CPD-specific antibody TDM-2 (MBL International) (diluted at 1:2000 with PBS) was added and incubated for 30 min at 37°C. Horseradish peroxidase-labeled goat anti-mouse IgG (Novogen) (diluted at 1:1000 with PBS) was added and incubated for 30 min at 37°C. Then 100 ml of substrate solution (8 mg o-phenylenediamine, 4 µl H_2O_2, 20 ml citrate phosphate buffer) was added to each well and incubated for 30 min at 37°C. The reaction was stopped by the addition of 50 µl of 2 M H_2SO_4 and the absorbance was measured at 492 nm using a spectrophotometer (UV-2401PC, Shimadzu).

Effect of UVR damage on viral capsid integrity and attachment ability. Both UV-B-treated and untreated cyanophage were digested with DNase I (0.1 mg ml^{-1}) and RNase (0.1 mg ml^{-1}) at room temperature for 1 h, then 1 ml of the sample was quantified by epifluorescence microscopy (Danovaro et al. 2001, Patel et al. 2007). The decrease in viral particles was inferred from the difference between the UV-treated and untreated viral samples. The percentage decrease of viral particle was calculated as the destruction rate.

To test the attachment ability of cyanophages, 9.5 ml of host culture (about 10^8 cells ml^{-1}) was inoculated with 0.5 ml of UV-B-treated or untreated cyanophage for 1 h under red light. Cells were then removed by centrifugation at 5000 × *g* for 5 min and the viral particles in the supernatant were counted by epifluorescence microscopy. The percentage decrease in viral particles (denoted as the adsorption rate) was calculated as the ratio between the amount of cyanophage in the supernatant and the initial amount in the sample.

Plaque assay for determining total infectivity decay rate and photorepair rate. The calculation and measurement of the total infectivity decay rate and photorepair rate were carried out as described in our previous work (Cheng et al. 2007). Under red light, each of the 0.1 ml UV-B-irradiated and unirradiated samples were quickly mixed with 0.9 ml host cells (>10^7 cells ml^{-1}). Red polyethylene (PE) cellophane was used to screen a portion of the light from the white fluorescent tube, providing red light. The mixture was incubated for 1 h, then serially diluted and used for the plaque assay in Petri dishes. To study the total infectivity decay rate, the plates were cultured in red light until plaque formation occurred. For examination of photorepair, the plates were cultured under white light. The unirradiated control sample was exposed to both red and white light. Finally, titers of cyanophage were recorded and used for analysis. Under both red and white light, the amount of inactivated virus was calculated from the difference between the viral titers of the treatments and the control; the total infectivity decay rate was denoted as the ratio (%) between the amount of inactivated virus and the titer of the control. The photoreactivating level was calculated from the difference between the titers of UV-B-exposed cyanophage with or without photorepair treatment. The repair rate (the percentage of revived infectivity by photorepair) was denoted as the ratio (%) between the titer of photorepaired virus and the amount of inactivated virus.

Data analysis. All above treatments was conducted in triplicate and differences between the data were statistically analyzed with 1-way ANOVA using GraphPad Prism software (GraphPad Software).

RESULTS

UV-B induced DNA damage in cyanophage PP

As shown in Fig. 1, the concentration of CPDs increased rapidly and reached a maximum in all 3 treatments. An increase in radiation exposure dose caused no significant increase in CPD levels ($p > 0.05$, ANOVA). The maximum CPD concentration, however, was significantly different in the 3 UV-B treatments ($p < 0.05$, 1-way ANOVA) and was positively correlated with radiation intensity. These results indicate that the DNA damage in cyanophage PP was UV-B intensity-dependent, not exposure dose-dependent.

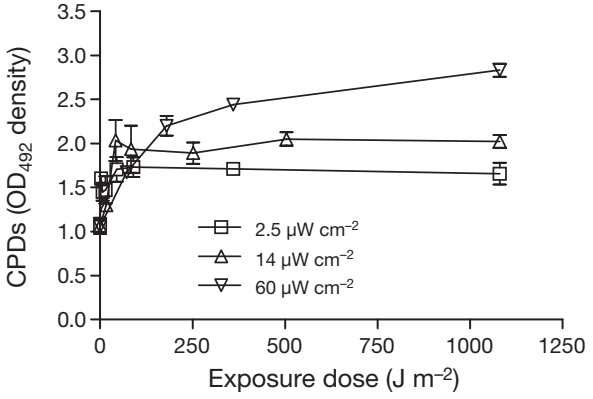

Fig. 1. DNA damage in UV-B-exposed cyanophage PP, represented by mean (±SD) cyclobutane pyrimidine dimer (CPD) concentration: 3 UV-B irradiation intensities are shown

UV-B-induced capsid destruction and decreased adsorption ability of cyanophages

UV-B radiation caused rapid viral destruction as shown in Fig. 2. The destruction rate increased rapidly when the exposure dose was below 45 J cm^{-2} and then remained stable as the exposure dose increased. The final destruction rate ranged from 14.36 to 18.92% depending on the radiation intensity. The adsorption rate decreased quickly at low exposure dose and showed no significant decrease when the exposure dose increased (Fig. 3). The final adsorption rate of UVR-treated cyanophage ranged from 36.89 to 41.16%, which was significantly lower than the control (61.23%). Both the final destruction rate and adsorption rate showed no significant difference among the 3 treatments ($p < 0.05$, 1-way ANOVA).

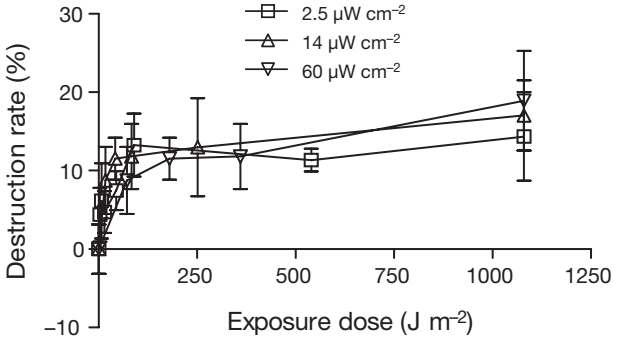

Fig. 2. Mean (±SD) destruction rate of UV-B-exposed cyanophage PP particles: 3 UV-B irradiation intensities are shown

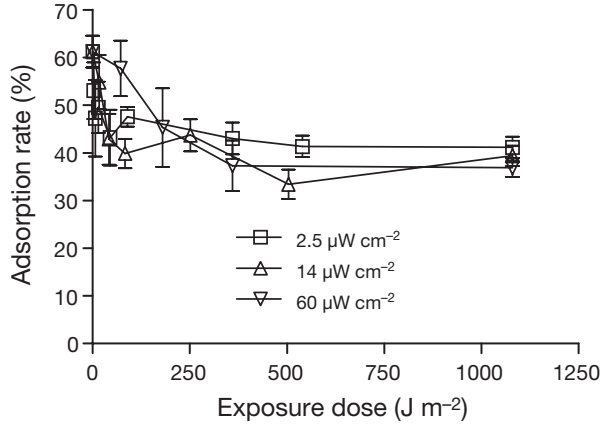

Fig. 3. UV-B-induced changes in attachment ability of cyanophage PP, represented as mean (±SD) adsorption rate: 3 UV-B irradiation intensities are shown

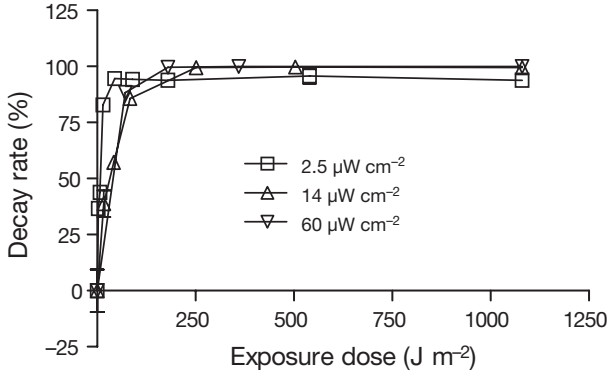

Fig. 4. Total infectivity decay rate (mean ± SD) of UV-B-exposed cyanophage PP, calculated by plaque assay: 3 UV-B irradiation intensities are shown

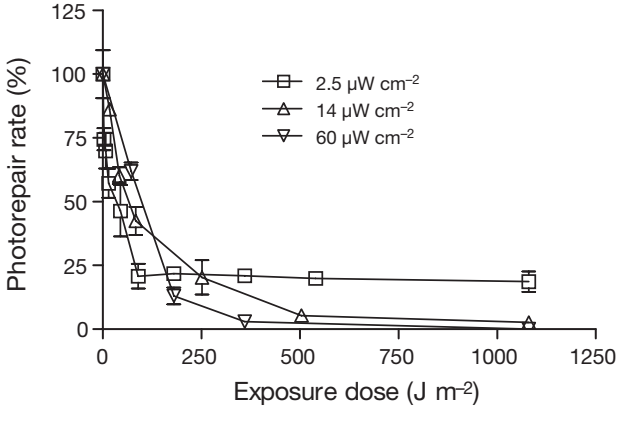

Fig. 5. Mean (±SD) photorepair rate of UV-B-damaged cyanophage PP: 3 UV-B irradiation intensities are shown

UV-B-induced total infectivity decay rate of cyanophages and photorepair rate in host cyanobacteria

The results of the plaque assay showed that UV-B radiation caused rapid inactivation of cyanophages at different radiation intensities (Fig. 4). The total infectivity decay rate peaked rapidly, and ranged from 93.46 to 99.99%, with no significant difference among the 3 treatments ($p > 0.05$, 1-way ANOVA).

UV-damaged cyanophages could be partially photorepaired by the host (Fig. 5). The repair rate calculated by plaque assay decreased with increasing exposure dose. The repair rate differed among the different treatment groups and was negatively correlated with UV-B intensity ($p < 0.05$).

DISCUSSION

UV-B radiation results in DNA damage through the formation of CPDs (Hader & Sinha 2005). At a given radiation intensity, DNA damage reaches saturation within a few minutes. Ronto et al. (2002) proposed a kinetic stochastic radiation model to explain this phenomenon in bacteriophage T7. In this model, it was assumed that the damaged sites could revert by absorption of UV photons, and that saturation represented the equilibrium between UV damage and UV photoreversion. However, this model cannot explain the differences in DNA damage caused at different UV intensities, as observed in our studies. We suggest that the secondary and tertiary structural changes to the viral DNA caused by UV-B radiation render the DNA resistant to further damage. It has been demonstrated that UV-induced lesions can distort the DNA helix (Wang & Taylor 1991, Kim et al. 1995). The UVR damage at a given base is influenced by the flexibility of the DNA and the base distribution. CPD formation is less frequent when there is bending of the DNA towards the minor groove (Pehrson & Cohen 1992). Besides, UV-induced DNA cross links can result in a tightly coiled DNA conformation (Pehrson & Cohen 1992). All these structural changes could result in the protection of some potentially damageable sites, such that only higher radiation intensities can penetrate to the protected sites and cause further damage.

Similar to the changes in CPDs, the decay rate in our study did not increase with longer radiation exposure dose. This phenomenon is called 'tailing' and has been observed in several other viruses (Maier et al. 1995, Craik et al. 2000). Tailing normally starts after at least 99% of the initially available micro-organisms have been inactivated, and the phenomenon is observed to a larger extent in the more UV-susceptible micro-

organisms (Hijnen et al. 2006). The cause of tailing is still unclear. We suggest that it may be determined by the extent of DNA damage, because the decay rate in our study was significantly correlated with the CPD concentration ($p < 0.01$). Inactivated cyanophages could be partially photoreactivated, and the final repair rates were different and closely related to the DNA damage level. This result further confirmed that the DNA damage was UV intensity dependent.

Our results also demonstrate that UV-B radiation could directly degrade the viral particles and could affect cyanophage adsorption, suggesting that UVR causes damage or structural changes of viral capsid proteins, especially the attachment proteins. UV-induced damage has been observed in the delta protein, an attachment protein which is analogous to VP4 of coxsackievirus and is responsible for viral adsorption (Miller & Plagemann 1974). Viral capsid structural damage and attachment ability loss caused by UVR has also been reported for several RNA viruses, such as human picornaviruses, feline calicivirus (FCV) and poliovirus (De Sena & Jarvis 1981, Nuanualsuwan & Cliver 2003). UV-damaged PV-1 (type I poliovirus) and FCV entirely lost attachment ability, indicating that inactivation required a conformational change of only a few receptor attachment sites to prevent attachment of the whole inactivated virus particle (Nuanualsuwan & Cliver 2003). However, our results indicate that only some of the damaged cyanophage PP lost attachment ability. This is similar to observations made with UV-damaged hepatitis A virus (HAV). These differences in loss of attachment ability are assumed to be due to differences in the capsid (especially the receptor attachment site) (Nuanualsuwan & Cliver 2003). Besides, results obtained by De Sena & Jarvis (1981) indicate that pH and ionic compounds could affect the extent of UV damage to the attachment ability of PV-1. Taken together, these results suggest that more work needs to be performed to study UV damage to viral capsids.

The phenomenon of UVR-influenced protein-DNA (and RNA) cross-linking has also been reported for RNA viruses, single-stranded DNA viruses and double-stranded DNA viruses (Francke & Ray 1972, Miller & Plagemann 1974, Ronto et al. 2002). A significant decrease in the yield of photoproducts has been detected in isolated T7 DNA and in heated phage compared to intraphage DNA (Ronto et al. 2002). The yield of photoproducts was similar in B, C-like and A conformational states of isolated T7 DNA, indicating that a conformational switch in the DNA was not the decisive factor in photoproduct formation. The authors suggested that the bound phage proteins induced an alteration in DNA structure that resulted in an increased rate of photoproduct production of adjacent bases in intraphage T7 DNA (Ronto et al. 2002). This supports the hypothesis that the DNA secondary and tertiary structure affects the formation of photoproducts, and also infers that a synergistic effect of UVR damage may exist between DNA and proteins in some viruses.

UV-induced DNA damage has been thought to play a major role in viral inactivation. However, our results have shown that direct viral particle destruction and the decrease in viral attachment ability caused by UVR also play important roles in the viral decay process. According to our results, different UVR intensities resulted in 14.36 to 18.92% viral particle destruction and 20.07 to 24.34% adsorption decrease. Since these results were acquired through epifluorescence microscopy, and only the completely destroyed particles could be distinguished, capsid protein damage that had no effect on viral integrity and adsorption could not be detected. Our results may, therefore, underestimate the capsid damage caused by UV-B. According to the hypothesis of UV-B toxicity thresholds, certain species possess tolerance thresholds to UVR (Pandelova et al. 2006). The threshold is determined by the repair capacity of the species, and radiation exceeding the threshold may be lethal. In the case of aquatic viruses, UV-B toxicity thresholds are determined both by viral resistance and host repair capacity. Viruses themselves have no repair system, and the toxicity threshold may be largely determined by viral resistance, especially capsid resistance. As the loss of attachment ability results in the loss of viral infectivity, solar radiation-induced capsid damage may play an important role in viral decay and survival, a fact that has previously been underestimated or ignored.

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