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NOTE

Solar UV radiation does not inactivate marine birnavirus in coastal seawater

Shin-Ichi Kitamura[1,2,*], Shin-Ichiro Kamata[1], Shin-ichi Nakano[1], Satoru Suzuki[1]

[1]Center for Marine Environmental Studies (CMES), Ehime University, Matsuyama 790-8577, Japan
[2]*Present address:* Department of Aqualife Medicine, Yosu National University, San 96-1 Dunduk-dong Yeosu, Jeollanam-do 550-749, Korea

ABSTRACT: We examined the inactivation kinetics of marine birnavirus (MABV) in a coastal sea, in seawater samples collected from 50 cm depth. MABV was added to both natural and autoclaved seawater at a concentration of $6 \times 10^{6.43}$ $TCID_{50}$ (50% tissue culture infectious dose) ml^{-1}, put in dialysis tubes and incubated at the original depth. The inactivation of MABV by solar UV radiation was examined using light and dark tubes. The infectivity titer of MABV was measured by the $TCID_{50}$ method using CHSE-214 cells. Virus infectivity in natural seawater decreased quickly and was below the detection limit by 270 min in both light and dark conditions; however, virus infectivity was maintained in the autoclaved seawater until 420 min. These results suggest that the loss of virus infectivity is not caused by sunlight UV radiation.

KEY WORDS: Ultraviolet · Birnavirus · Seawater · Infectivity · Inactivation

INTRODUCTION

Marine birnavirus (MABV), a member of the *Birnaviridae*, is an icosahedral unenveloped virus with a genome consisting of 2 segments of double-stranded RNA (Dobos et al. 1979). MABV was first isolated from diseased yellowtail *Seriola quiqueradiata* in Japan in 1985 (Sorimachi & Hara 1985). Similar birnaviruses have been isolated from shellfish and other cultured and wild fish from around the world (Sohn et al. 1995, Cutrin et al. 2000, Isshiki et al. 2001). Many of the birnavirus isolates were genetically similar (Kitamura et al. 2000), suggesting that all isolates belong to the MABV group. MABVs have a broad host range in various marine organisms (Cutrin et al. 2000, Isshiki et al. 2001).

The transmission mode of MABVs has not been fully defined. MABVs have previously been detected by PCR from coastal and open seawater (Kitamura & Suzuki 2000, Suzuki et al. 2001), which suggests that MABV can be reserved in seawater as a cell-free form. In addition, yellowtail ascites virus (YAV) and viral deformity virus (VDV), which belong to the MABV group, could infect the host organism by bath challenge (Maeno & Nakajima 1997), suggesting that MABVs horizontally infect via seawater. Thus, it is thought that MABVs released from affected fish go on to infect healthy fish. However, we could not isolate MABV from seawater samples (Kitamura & Suzuki 2000, Suzuki et al. 2001). In order to clarify why it was not possible to isolate MABV from seawater, the inactivation process of MABV should be understood. To investigate the infection route of MABV via seawater, it is also important to know the time needed for its inactivation.

It has been reported that virus infectivity is reduced by exposure to solar ultraviolet (UV) radiation (Suttle & Chen 1992, Noble & Fuhrman 1997), high temperature (Chung & Sobsey 1993) and biological factors in aquatic environments (Yoshimizu et al. 1986a, Kamei et al. 1987). The goal of the present study was to exam-

*Email: tachiuomaster@hotmail.com

ine the inactivation kinetics and discuss whether sunlight can inactivate MABV in coastal seawater in summer.

MATERIALS AND METHODS

The chinook salmon embryo cell line (CHSE-214) was used for MABV propagation and assay of virus infectivity. Cells were grown at 20°C in Eagle's minimum essential medium (MEM, Nissui), which contained 10% fetal bovine serum (FBS). MABV Strain JPO-97 isolated from the Japanese pearl oyster *Pinctada fucata* (Kitamura et al. 2000) was used in this study. The virus was inoculated to monolayer cells of CHSE-214 in 75 cm^2 flasks (Corning). When the cytopathic effect (CPE) reached 100% of the monolayer, the culture fluid was harvested. Cells were freeze-thawed twice to obtain the cell-associated viruses. The culture fluid was centrifuged at 2800 × *g* at 4°C for 20 min to remove cell debris, and supernatant was used as a virus sample. The infectivity titer was measured by the 50% tissue culture infectious dose ($TCID_{50}$), calculated by Reed & Muench (1938) using a 96-well tissue culture plate (Corning).

UV intensity was measured using a PUV-500 (Biospherical Instruments) on 22 and 23 August 2001. The weather on each day was clear, and UV transmission was calculated using the following formula:

$$\% \text{ transmittance} = (I/I_0) \times 100$$

where I_0 = intensity of incident monochromatic light at the surface and I = final intensity of transmitted radiation after passing through the seawater.

The present study was conducted in Uchiumi Bay, west coast of Ehime Prefecture, Shikoku Island, Japan, on 25 August 2001. Seawater was collected from 50 cm depth using a Niskin sampler, and a portion of the seawater sample was autoclaved. A total of 50 ml of natural, autoclaved seawater was poured into sterilized dialysis tubes (Spectrum; MWCO: 3500, molecular mass cut-off of 3500 Da), and 3 ml of the virus solution was added to each tube at a concentration of $10^{8.43}$ $TCID_{50}$ ml^{-1}. To prevent breakage, each tube thus prepared was placed in a transparent polycarbonate bottle with many holes in it to allow transmittance of UV. In order to know effect of UV radiation, these samples were divided into 2 groups: one exposed to sunlight, the other covered with black cloth to block UV radiation. Thus, we had 4 treatments: natural, light; natural, dark; autoclave, light; autoclave, dark. Each treatment group was performed in triplicate. These treatments were incubated at 50 cm depth. A subsample was taken from each tube at 20 or 50 min intervals, immediately frozen in liquid nitrogen and then stored at –80°C until use. Before measuring the infectivity, each subsample was filtered through a 0.45 µm pore filter (Millipore).

Table 1. UV transmission rate at 50 cm depth

Wavelength (nm)	% transmission relative to surface
308	74.97
320	73.58
340	78.21
380	82.33
PAR	82.23

RESULTS AND DISCUSSION

Water temperature during the experiment ranged between 27.2 and 28.2°C at 50 cm depth. UV transmission at 50 cm depth was higher than 70% of the intensity at the surface (Table 1).

The infectivity titer of MABV declined over time in all treatments (Fig. 1). The mean initial infectivity titer was $10^{3.80}$ $TCID_{50}$ ml^{-1}, even though the original virus solution had a titer of $6 \times 10^{6.43}$ $TCID_{50}$ ml^{-1}, suggesting non-specific adsorption of the virus to the tube. Virus infectivity in the natural seawater markedly declined after 120 min incubation in both light and dark treatments. The infectivity titers declined below the detection limit (10^2 $TCID_{50}$ ml^{-1}) after 270 min. By contrast, in the autoclaved seawater virus infectivity was maintained even after 420 min.

Until now, it has been reported that viruses, especially phages, can be inactivated by solar UV radiation in environmental seawater (Suttle & Chen 1992, Noble & Fuhrman 1997). The loss of the MABV titer was similar in both the light and dark treatments, although our experiment showed that more than 70% of the UV radiation was detected at 50 cm depth. This result suggests that MABV was resistant to solar UV radiation. One type of birnavirus, infectious pancreatic necrosis virus (IPNV), has been reported to be inactivated at 1.0 to 1.5×10^5 µW s^{-1} cm^{-2} (Yoshimizu et al. 1986b). Øya & Rimstad (2001) reported that IPNV is more resistant to UV radiation than both infectious salmon anaemia virus and viral haemorrhagic septicaemia virus. Liltved et al. (1995) also reported that IPNV was more resistant to UV irradiation (1.99 to 1.25×10^5 µW s^{-1} cm^{-2} was needed for 99.9% inactivation) than *Aeromonas salmonicida*, *Vibrio anguillarum* and *Yersinia ruckeri*. Together, these reports suggest that birnavirus is resistant to UV radiation, even under laboratory conditions. Our data indicate that solar UV radiation at 50 cm depth was not strong enough to inactivate MABV. Resistance of birnaviruses against UV radiation may be due to the small size of the virion.

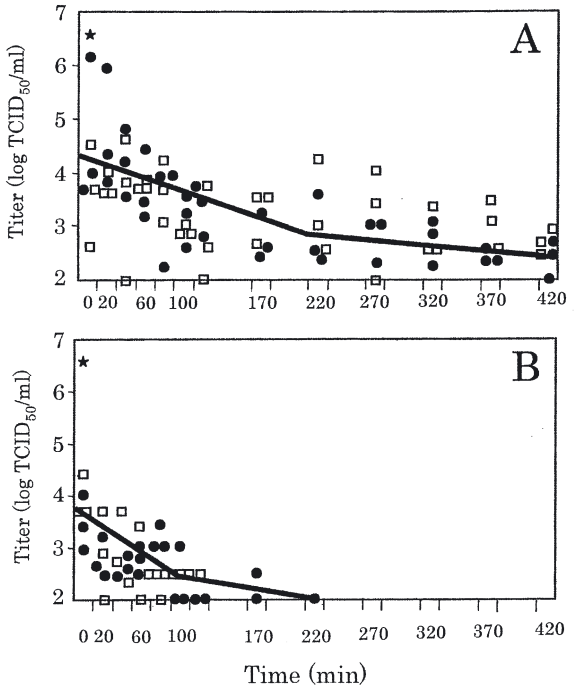

Fig. 1. Infectivity titer of marine birnavirus (MABV) suspended in (A) autoclaved seawater and (B) natural seawater. (●) Dark condition; (□) light condition; (★) original virus titer. Detection limit of the infectivity titer was 10^2 $TCID_{50}$ ml^{-1}

MABV is stable against heating at 56°C for 30 min (Sorimachi & Hara 1985). Mortensen et al. (1998) reported that IPNV isolated from the scallop *Pecten maximus* was stable at 30°C for 10 d under laboratory conditions. These findings suggest that birnaviruses are stable at high temperatures. Our experiment was performed at temperatures ranging from 27.2 to 28.2°C at a depth of 50 cm. Thus, neither solar UV radiation nor temperatures in this range caused the decline in virus infectivity.

Biological factors such as protease, anti-viral substance and other microbes are thought to be virus-inactivating factors. Marine bacteria are known to produce extracellular protease (Odagami et al. 1994). Proteolytic enzymes degrade organic matter in ocean ecosystems (Davey et al. 2001), which is important in the cycling of organic matter, and these enzymes are also thought to decay viruses in the ocean. Yoshimizu et al. (1986a) reported that culture filtrate of *Pseudomonas* isolates from a salmonid hatchery reduced the infectivity titer of infectious hematopoietic necrosis virus (IHNV) to a non-detectable level within a 3 d incubation, and the virus-inhibiting factor produced by *Pseudomonas* was inactivated by autoclaving. Our results may be caused by a similar factor.

Environmental microorganisms play an important role in the inactivation of viruses in seawater. Kamei et al. (1987) reported a similar phenomenon, in which the infectivity of IPNV was reduced by more than 2 orders of magnitude after 7 d in natural seawater collected in summer, whereas the virus was stable even at 14 d in seawater that was autoclaved or filtered. This loss of infectivity has also been documented in other articles. Fujioka et al. (1980) reported that poliovirus was not detected in natural seawater after a 1 d incubation at 24°C, but was stable even after 3 d in autoclaved or filtered seawater. Although MABV is relatively stable compared to other viruses against UV and high temperature, our results showed that reduction of the infectivity of MABV occurred within 3 h in natural seawater. A high level of anti-viral bacteria and/or degrading enzymes might have contributed to this reduction.

We have reported that MABV can be detected in both coastal areas and the open ocean (Kitamura & Suzuki 2000, Suzuki et al. 2001), suggesting that even if infectivity was lost, genomic RNA covered by a capsid protein is stable in seawater. This study could explain why MABV can be detected by PCR but not by the cell-culture method (Kitamura & Suzuki 2000, Suzuki et al. 2001). This present study suggests that MABV can infect host organisms for <220 min before the infectivity is lost. Since fish and shellfish are generally cultured at high densities in net cages, 220 min is enough time for MABV to infect other individuals. MABV might be able to transfer horizontally to different species, or many individuals of the same species before inactivation. Finally, we conclude that UV irradiation does not necessarily inactivate MABV in natural seawater.

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