THE INACTIVATION OF HEPATITIS A VIRUS AND OTHER MODEL VIRUSES BY UV IRRADIATION

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ABSTRACT

Ultraviolet light is an attractive alternative to chemical disinfection of water, but little is known about its ability to inactivate important waterborne pathogens such as hepatitis A virus. Therefore, the sensitivity of HAV strain HM-175, coxsackievirus type B-5, rotavirus strain SA-II, and bacteriophages MS2 and φX174 to ultraviolet radiation of 254 nm wavelength in phosphate buffered water was determined. Purified stocks of the viruses were combined and exposed to collimated UV radiation in a stirred reactor for a total dose of up to 40 mW sec/cm². Virus survival kinetics were determined from samples removed at dose intervals. The 4 log₁₀ (99.99%) inactivation doses for HAV, CB5, SA-II and φX174 were 16, 29, 42 and 9 mW sec/cm², respectively. MS2 exhibited the greatest resistance in buffered water with less than a 1 log₁₀ reduction observed after exposure to 25 mW sec/cm². A 15 mW sec/cm² exposure induced a 7 log₁₀ reduction of φX174, while inactivation of HAV, CB5 and SA11 was intermediate, with at least 3 log₁₀ reductions occurring after a 20 mW sec/cm² exposure. The results of these experiments indicate that UV radiation can effectively inactivate viruses of public health concern in drinking water.

KEY WORDS

Disinfection; ultraviolet light; hepatitis A virus; coxsackievirus B5; rotavirus SA-II; coliphages; MS2; φX174

INTRODUCTION

Disinfection continues to be one of the most important treatment barriers against waterborne disease. However, concerns about the production of chlorinated organics and questions pertaining to the efficiency of chemical disinfectants have led to consideration of alternative disinfection practices. Additionally, it is becoming evident that conventional water treatment practices and the use of traditional bacteriological indicator organisms provide insufficient protection against contamination by non-bacterial agents. These problems have prompted the search both for more effective disinfectants as well as for more appropriate indicators of water quality. The objectives of this study were to evaluate the application of UV radiation against a battery of enteric viruses and bacteriophages in an attempt to define the comparative inactivation kinetics of these viruses in water.

MATERIALS AND METHODS

Animal Viruses

Hepatitis A virus (HAV) strain HM-175 was propagated and assayed in FRhK-4 cells (Cromeans et al., 1987). Coxsackievirus B5 and rotavirus SA-II were propagated and assayed in BGMK and MA104 cell lines (Sobsey et al., 1978; Smith et al., 1979). Assays of all animal viruses were performed using the plaque assay method. Monolayers were...
infected at a multiplicity of approximately 0.1 PFU/cell with plaque purified seed stock. The lysates of 100% infected cultures were fluorocarbon extracted and centrifuged at 6000 x g for 30 min. The supernatant was ultracentrifuged at 95000 x g for 2 hours to pellet the virus. Viruses were resuspended in phosphate buffered saline, and monodisperse stocks were produced by filtration through Tween-80-treated 0.08 µm polycarbonate filters (Nuclepore, Inc., Pleasanton, CA).

Bacterial Viruses
Bacteriophages were propagated by the double (top) agar layer (DAL) plaque technique. Phage MS2 was grown as confluent lysis DAL plates on E. coli C3000 top agar and was scraped and suspended in small volumes of phosphate buffered saline (PBS). Phage φX174 was grown in E. coli C by the same method. Cell debris was removed by chloroform extraction and low speed centrifugation at 5000 x g for 15 min. Recovered phage was pelleted at 90,000 x g for 4 hours at 5°C. Pellets were resuspended in PBS and filtered through Tween-80 treated 0.08 µm Nuclepore filters.

EXPERIMENTAL PROTOCOL
The UV apparatus consists of a bank of 4 germicidal lamps which were suspended 38 cm above the exposure area. Viruses were irradiated in 60x15 mm plastic petri dishes containing 10 mL PBS and were stirred slowly on a magnetic stir plate. Collimated radiation was measured with an International Light IL500 radiometer using a detector for incident intensity at 254 nm wavelength. Dose was computed as the product of radiation intensity and time (in minutes), and was calculated according to the following equation:

\[ D = I_o \times (1 - e^{-aL}) / aL \]

where \( I_o \) = incident intensity; \( a \) = absorbance per cm suspension, and \( L \) = path length (cm)

Dose is recorded in units of mW sec/cm², and samples were withdrawn at 5 unit intervals up to the maximum of 40 mW sec/cm². Exposure times were controlled by selectively blocking radiation with a screen before and after the desired exposure times. Experiments were performed in duplicate, and calibration curves of log survival versus dose were used to determine dose and intensity. Geometric means of the proportionate \( 10^\log_{10} \) reductions of virus were then computed.

RESULTS
The results of the UV inactivation experiments demonstrate that UV radiation at a dose of 25 mW sec/cm² is capable of inactivating 99.9% of all test viruses with exception of bacteriophage MS2 (Figure 1). All test viruses exhibited essentially first order
inactivation kinetics with the exception of rotavirus SA-II, which exhibited a slower inactivation rate at higher doses. Bacteriophage φX174 was inactivated the most rapidly, undergoing almost 7 log_{10} inactivation by a dose of 15 mW sec/cm^2. In contrast, bacteriophage MS2 exhibited less than 1 log_{10} inactivation at almost twice the dose. Reductions of the animal viruses were intermediate, with HAV exhibiting the greatest sensitivity. Coxsackievirus B5 and rotavirus SA-II were reduced by approximately 3 log_{10} with an exposure of 25 mW sec/cm^2, while HAV was reduced by 4 log_{10} using 15 mW sec/cm^2.

DISCUSSION

The considerable resistance of coliphage MS2 to UV inactivation compared to the other test viruses is striking and not readily explained. It may be related to the small size of the target genomic nucleic acid (only 3.57 kb compared to the next smallest genome of ca 5.36 kb for φX174), or other features of virion structure and organization.

The considerable relative sensitivity of φX174 to UV inactivation, a single stranded DNA bacteriophage, is consistent with observations noted in previous studies (Winkler, 1964; Furuse et al., 1967). The relatively rapid rate of inactivation may be related to the fact that φX174 was the only DNA virus within the test group, and its DNA occurs in a closed loop, in contrast with the linear genomes of the other model viruses tested. The pyrimidine bases of viral DNA include cytosine and thymine, and it has been reported (Jagger, 1967) that thymine dimers form more readily during UV exposure than do the uracil dimers of UV-exposed RNA viruses. φX174 exhibits a high thymine content (31%) relative to the uridine content of bacteriophage MS2 (25%).

The inactivation curves observed for the five test viruses suggest that hepatitis A virus is significantly (p<.01) less resistant to UV radiation than the other animal viruses, while the responses of coxsackievirus B5 and rotavirus SA-II could not be distinguished significantly from one another. The sensitivity of hepatitis A virus may be attributable to its relatively high uracil content (33%), as uracil hydration has been observed as an effect of UV light on polynucleotide chains (McLaren & Shugar, 1964). The relative resistance of rotavirus may be attributed to the fact that SA-II is a double stranded RNA virus and its genome size (>15,000 nucleotides) is considerably larger than that of HAV, rendering it less sensitive to UV inactivation due to the greater number of hits required to destroy its larger genome. Double stranded nucleic acids exist in a highly ordered state due to hydrogen bonding within the helices and exhibit lower absorbance of radiation in the UV range than their single stranded counterparts (Patrick and Rahn, 1976). The complementary strands of double stranded viruses may also provide sufficient redundancy to allow for additional resistance to physical and chemical inactivation.

A tailing, or retardant die-off effect was observed for rotavirus, where a slight decline in the inactivation rate occurred after a total dose of 17 mW sec/cm^2. These results are consistent with retardant inactivation kinetics observed in chlorine inactivation studies using reovirus and poliovirus (Hill et al., 1971). Similar tailing effects have been attributed to aggregation of viral particles (Floyd et al., 1979).

Sensitivity to UV inactivation may also be influenced profoundly by multiplicity reactivation phenomena. Multiplicity reactivation of partially damaged viruses may account for differences in UV sensitivity since stressed or damaged viruses may be unable to initiate viral infection of cells without the aid of companion viruses. Therefore large numbers of partially damaged viruses may be required to cause visible plaques in cell culture assays of viruses. Multiplicity reactivation phenomena may play a greater role in conferring UV resistance to viruses which exhibit a strong tendency to aggregate spontaneously, such as the coxsackieviruses. Initial infection of target cells is more likely to involve coinfection by several viruses simultaneously if the viruses are initially present in aggregates (Rohwer and Gajdusek, 1980).

The recommended dose of 16 mW sec/cm^2 for UV disinfection of water would have resulted in a reduction of at least 99% of all viruses in this study with the exception of MS2. However, the viruses were highly purified and were present in a solution which exhibited minimal absorption of UV radiation. In waters of high turbidity or color, and with particle-associated organisms, disinfection efficiency is predicted to be much
weaker. Furthermore, if disinfection processes must achieve a greater degree of virus inactivation, such as 99.99%, a dose of 16 mW sec/cm² would not achieve this level for HAV, CBS, rotavirus, and perhaps other enteric viruses. Therefore, larger doses of UV may be required to achieve extensive virus reductions and to provide a sufficient margin of safety.

REFERENCES


