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Wavelength Dependent UV Inactivation and DNA Damage of Adenovirus as Measured by Cell Culture Infectivity and Long Range Quantitative PCR

Sara E. Beck,[†] Roberto A. Rodriguez,[‡] Karl G. Linden,*[,†] Thomas M. Hargy,[§] Thomas C. Larason,[∥] and Harold B. Wright[⊥]

[†]Department of Civil, Environmental, and Architectural Engineering, University of Colorado Boulder, UCB 428, Boulder, Colorado 80309, United States

[‡]Environmental and Occupational Health Sciences, School of Public Health − El Paso Regional Campus, University of Texas-Health Science Center at Houston, 1101 N. Campbell St., Room 414, El Paso, Texas 79902, United States

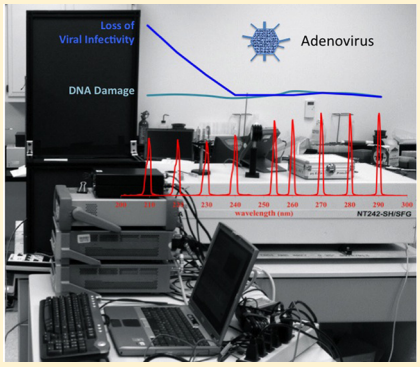
[§]Tetra Tech Clancy Environmental, 20 Mapleville Depot, Saint Albans, Vermont 05478, United States

[∥]National Institute of Standards and Technology (NIST), 100 Bureau Dr., Gaithersburg, Maryland 20899, United States

[⊥]Carollo Engineers, 12592 West Explorer, Suite 200, Boise, Idaho, 83713, United States

Supporting Information

ABSTRACT: Adenovirus is regarded as the most resistant pathogen to ultraviolet (UV) disinfection due to its demonstrated resistance to monochromatic, low-pressure (LP) UV irradiation at 254 nm. This resistance has resulted in high UV dose requirements for all viruses in regulations set by the United States Environmental Protection Agency. Polychromatic, medium-pressure (MP) UV irradiation has been shown to be much more effective than 254 nm, although the mechanisms of polychromatic UV inactivation are not completely understood. This research analyzes the wavelength-specific effects of UV light on adenovirus type 2 by analyzing in parallel the reduction in viral infectivity and damage to the viral genome. A tunable laser from the National Institute of Standards and Technology was used to isolate single UV wavelengths. Cell culture infectivity and PCR were employed to quantify the adenoviral inactivation rates using narrow bands of irradiation (<1 nm) at 10 nm intervals between 210 and 290 nm. The inactivation rate corresponding to adenoviral genome damage matched the inactivation rate of adenovirus infectivity at 253.7 nm, 270 nm, 280 nm, and 290 nm, suggesting that damage to the viral DNA was primarily responsible for loss of infectivity at those wavelengths. At 260 nm, more damage to the nucleic acid was observed than reduction in viral infectivity. At 240 nm and below, the reduction of viral infectivity was significantly greater than the reduction of DNA amplification, suggesting that UV damage to a viral component other than DNA contributed to the loss of infectivity at those wavelengths. Inactivation rates were used to develop a detailed spectral sensitivity or action spectrum of adenovirus 2. This research has significant implications for the water treatment industry with regard to polychromatic inactivation of viruses and the development of novel wavelength-specific UV disinfection technologies.



INTRODUCTION

Adenovirus is an enteric virus associated with respiratory and gastrointestinal illness in humans, most virulently affecting immunocompromised individuals. It has been associated with waterborne disease outbreaks[1] and has been detected in treated drinking water.[2] As a known public health risk subject to future regulations, it is one of few microbial contaminants listed on the U.S. Environmental Protection Agency's Contaminant Candidate List (CCL 3). Relative to other pathogens, adenovirus demonstrates strong resistance to UV light. When disinfecting with monochromatic, low-pressure (LP) ultraviolet (UV) light at 253.7 nm, 4-log inactivation requires a dose between 120 mJ/cm^2 to 170 mJ/cm^2, four times greater than that required for inactivating other enteric viruses, including echovirus, coxsackievirus, and poliovirus.[3−6] Given this resistance, adenovirus governs the U.S. Environmental Protection Agency regulations for virus inactivation. The Long-Term 2 Enhanced Surface Water Treatment Rule (LT2), which requires 4-log inactivation of viruses, stipulates a minimum UV dose of 186 mJ/cm^2, based on statistical analysis of empirical results of adenovirus inactivation by LP UV light.[7−9] For groundwater treatment, the US EPA Groundwater Rule declared that UV is not a sufficient standalone treatment for viruses unless adequate inactivation is demonstrated through a field-scale challenge test.[10]

Although adenovirus has demonstrated a resistance to LP UV light, polychromatic UV light from medium-pressure (MP) mercury

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vapor lamps is two to four times more effective.[4,11] A MP UV dose of 40 mJ/cm^2 to 80 mJ/cm^2 is necessary for 4-log inactivation, where germicidal dose is calculated using the UV absorbance of DNA.[12] The high resistance of adenovirus to LP UV could be the result of nucleic acid damage being repaired during infectivity assays. When adenovirus was assayed using a cell line with limited DNA repair capability (XP17BE) the doses for 4-log inactivation using LP and MP UV lamps were as low as 57 mJ/cm^2 and 42 mJ/cm^2, respectively.[13] Although more effective than LP UV light, the mechanisms behind the enhanced efficacy of MP UV light have not been well characterized. UV light is generally subdivided into UVC (100−280 nm), UVB (280−315 nm) and UVA (315−400 nm). The region between 200 and 300 nm is directly absorbed by DNA and therefore considered the germicidal UV region. Kuluncsics et al. found that UVC is 10^5 times more effective than UVA at inducing cyclobutane pyrimidine dimers (CPDs), the dominant form of UV-induced DNA damage.[14] Besaratinia et al. determined that the formation of CPDs and other photodimeric lesions is dependent upon wavelength.[15] In both previous studies, the DNA was isolated prior to exposure to UV light, limiting the potential insights into interactions between DNA and proteins that could occur in vivo with a water treatment application.

Measuring nucleic acid damage gives insight into the mechanisms involved in UV inactivation. DNA damaged by UV light inhibits Taq DNA polymerase progression through the template DNA strand.[16] The longer the DNA strand, the higher the possibility of the polymerase to encounter damage. Therefore, polymerase chain reaction (PCR) assays with the ability to amplify long fragments of DNA allow for greater sensitivity by enabling the detection of biologically relevant DNA damage.[17,18] When assaying equal amounts of DNA using PCR, a reduction in amplification corresponds to DNA damage; the method is therefore a useful tool for quantifying damage to the viral genome.[19] A long-range quantitative PCR method was adapted for adenovirus and proposed as an alternative to cell culture for detecting adenovirus inactivation by UV light.[20] When direct DNA damage is measured, LP and MP UV light are equally effective at damaging the adenovirus genome despite their difference in efficacy at inactivating the virus as determined by cell culture assays.[19]

The goal of this study was to provide more insight into the fundamental mechanisms of adenovirus inactivation from polychromatic germicidal UV light by determining the impact of specific UV wavelengths in inactivating adenovirus, particularly at lower wavelengths (<240 nm). In this study, a tunable laser from the National Institute of Standards and Technology was used to isolate monochromatic wavelengths from 210 to 290 nm. At each wavelength, the viral infectivity and genome damage were analyzed to determine the spectral sensitivity of adenovirus and its genome and enhance our understanding of the link between UV-induced nucleic acid damage and loss of infectivity.

The resulting adenovirus action spectrum could be applied in the germicidal fluence calculation when using polychromatic UV light sources such as MP UV lamps for virus disinfection. The suggested practice for calculating UV dose involves weighting the average irradiance of the water sample by a generic DNA absorption spectrum (from *E. coli*), normalized to 254 nm to determine the average germicidal irradiance.[12] If this approach is valid, the response of an organism to MP UV light should coincide with its response to LP UV light, as is the case with *E. coli* and *Mycobacterium terrae*.[21,22] However, other research has suggested that the medium-pressure UV dose should be calculated based on the microbial or viral action spectrum as opposed to DNA absorption to account for non-DNA based damage, such as damage to viral proteins occurring from exposure to polychromatic light.[23,24] In this study, the MP UV dose calculation was adjusted to weight the average irradiance by action spectrum and the results were compared with the LP UV dose response.

To our knowledge, this is the first study analyzing the spectral sensitivity of DNA across the deep UVC spectrum, applying novel and precise irradiation techniques via a tunable laser for wavelength-specific UV inactivation in combination with analysis of spectral sensitivity of adenovirus on the molecular level. Understanding the wavelength-specific effects of UV light on nucleic acids can help us understand the effect of low-wavelength (<240 nm) UV light, which could have a significant impact on MP UV system validation and potentially encourage the use of MP UV systems for virus inactivation in groundwater. The research can also assist with the design of novel, tailored-wavelength UV disinfection technologies, combining, for example, light emitting diodes (LEDs) with specific output spectra.

MATERIALS AND METHODS

UV Irradiations. UV irradiations of adenovirus suspended in phosphate buffered saline (PBS) were conducted using a NT242 series Ekspla tunable laser provided by the National Institute of Standards and Technology (NIST, Gaithersburg, MD). The Ekspla incorporates a pulsed (1 kHz) Nd:YAG pump laser and an optical parametric oscillator (OPO), which provide the capability to tune the laser output between 210 and 2600 nm. Visible light from the laser that was coaligned with the UV wavelengths was either filtered by dielectric mirrors or diverted by aluminum mirrors and a prism from the optical path. A NIST compact array spectrometer (Instrument Systems CAS 140 CT) was used to verify that no visible light reached the samples. The full width at half-maximum (FWHM) bandwidth of the laser radiation was calculated at 0.04 at 300 nm emission and 0.07 at 210 nm. When measured with a Maya 2000 Pro spectrometer (Ocean Optics, Dunedin, FL), the FWHM varied between 1.1 at 210 nm and 1.7 at 300 nm; the wider FWHM is caused by the bandwidth of the Maya 2000 Pro. A figure of the NIST tunable laser emission at each wavelength is available in the Supporting Information (SI).

At wavelengths between 210 and 290 nm in 10 nm intervals, four collimated beam exposures were conducted to generate a dose response curve up to 3-log inactivation. Irradiance was measured at the water surface by a photodiode detector (IRD SXUV 100, Opto Diode Corporation, Thousand Oaks, CA) and precision aperture (SK#030483-1073, Buckbee Mears, Cortland, NY), both supplied by NIST. Average UV doses were determined as described in Bolton and Linden,[25] adjusting for reflection off the water surface, UV absorption (measured by a Spectronic Genesys 10uv spectrophotometer, Thermo Electron Scientific Instruments Corp, Madison, WI), depth of the water sample, as well as the nonuniform distribution of light across the sample surface. Beam divergence was assumed to be negligible from the laser diffuser, but measured and accounted for in the mercury lamp irradiations. Quiescently stirred samples of 5 mL (0.6 cm depth) were irradiated in 3.5 cm diameter Petri dishes. UV doses ranged from 8 mJ/cm^2 to 160 mJ/cm^2, depending on the wavelength. Laser irradiance varied across wavelengths between 10 μW/cm^2 and 300 μW/cm^2. For DNA-based analysis, two independent experiments were conducted to collect a replicate set of samples at each wavelength. Immediately after exposure and prior to the plaque assay, the irradiated samples

were stored at −80 °C. An aliquot of each sample from the −80 °C freezer was also shipped to University of Colorado Boulder for molecular DNA-based analysis.

Collimated beam exposures were also conducted to compare the adenovirus dose response to LP UV and MP UV light. UV doses with the low-pressure mercury vapor lamp (G12T6L, Atlantic Ultraviolet, Hauppauge, NY) were determined as described above. For the MP UV mercury vapor lamp (Rayox 1 kW, Calgon Carbon Corporation, Pittsburgh, PA), the UV dose accounts for the relative lamp emission of the light source (as measured by the Maya 2000 Pro spectrometer), the sensitivity of the radiometer used to measure irradiance (from radiometer calibration data), the absorbance and sample depth of the water, as well as a germicidal weighting. In this study, average irradiance was weighted germicidally with DNA absorption as is the common practice,[12] as well as with the adenovirus action spectrum, determined below, for comparison.

Cell and Virus Propagation and Enumeration. Adenovirus 2 (ATCC #VR-846) was propagated in A549 human lung carcinoma cells (ATCC #CCL-185). For UV-irradiated samples containing adenovirus, a most probable number (MPN) assay was performed.[26,27] An MPN result was determined using a spreadsheet based on the calculation methods given in Standard Methods.[28] Detailed information regarding cell and virus propagation and virus enumeration can be found in the SI.

Long Range and Quantitative Polymerase Chain Reaction Assay (LR-qPCR). A two-step long range quantitative polymerase chain reaction (LR-qPCR) procedure was used to detect UV damage to the viral genome.[20,29] In the first step, long range PCR was performed to amplify intact DNA fragments 1.1 kilobase pairs (kbp) long. In the second step, qPCR was used to quantify the products of the LR PCR. Detailed information of this two-step LR-qPCR approach is presented in the SI.

UV Absorption of DNA. Viral DNA from the adenovirus 2 stock (10^8 pfu/mL) was extracted as described above. The UV absorption was determined from 200 to 300 nm using a UV spectrophotometer (Nano Drop 1000, Thermo Fischer Scientific, Wilmington, DE) to compare with the spectral sensitivity of DNA measured with LR-qPCR. The ratio of UV absorption at 260 and 280 nm was 1.83, confirming the purity of the DNA.

Statistical Analysis. For the LR-qPCR analysis, a serial, 10-fold dilution curve was developed to correlate the PCR cross threshold value (Ct) with adenovirus concentration and measure changes in the log concentration of gene copies. The viral stock concentration in genome copies was determined using a calibrated real-time PCR for the adenovirus hexon gene described previously.[30] The calibration of the Hexon gene real-time PCR was performed as described previously.[20] The following linear equation was used for calculating $\log_{10}$ copies when using the LR-qPCR from adenovirus stock.

$$\log_{10}\text{copies} = -(0.3059 \times \text{Ct}) + 14.684 \; r^2 = 0.962 \quad (1)$$

Analysis of the relation between $\log_{10}$ copies and UV dose showed a linear response with no statistically significant curvature. Dummy variable regression analysis[31] showed that repeated UV dose−response curves at a given wavelength had the same slope ($p < 0.05$) but different intercepts. The kinetic constants for the relation between $\log_{10}$ copies and UV dose reported here, as well as their 95% confidence intervals, were obtained from the dummy variable regression analysis.

The log reduction of amplifiable DNA was calculated as

$$\log\text{reduction} = \log_{10}\text{copies}(D_0) - \log_{10}\text{copies}(D) \quad (2)$$

where $\log_{10}$ copies (D_0) is the PCR response of the unexposed sample predicted by the dummy variable regression analysis (i.e., the intercept) and $\log_{10}$ copies (D) is the PCR response obtained with a given UV dose, D.

The relationship between the log concentration of adenovirus and UV dose was also linear, showing no statistically significant curvature. The kinetic constant for the relationship between log (N) and UV dose was obtained from regression analysis. The log reduction of adenovirus was calculated as

$$\text{Log reduction} = \log(N_0) - \log(N) \quad (3)$$

where log (N_0) was the log concentration of the unexposed sample obtained from the regression analysis (i.e., the intercept), and log (N) is the measured log concentration.

Analysis of covariance (ANCOVA) was used to compare the linear regression for DNA damage with the linear regression for infectivity to determine if they were statistically similar ($p > 0.05$). Inactivation rate constants for infectivity and DNA damage were taken relative to their values at 253.7 nm to illustrate the action spectrum or spectral sensitivity of adenovirus and its DNA to UV light emission from a LP UV lamp. ANCOVA was also used to compare the LP UV and MP UV dose response given different MP UV fluence calculations.

RESULTS AND DISCUSSION

Inactivation of adenovirus 2 using LP UV light and the NIST laser at 253.7 nm are presented in Figure 1. For LP UV, the inactivation rate (k, cm^2/mJ) was 0.023 ($p = 4.4 \times 10^{-4}$) for cell culture infectivity and 0.022 ($p = 3.3 \times 10^{-9}$) for the 1.1 kbp fragments analyzed with the LR-qPCR assay. For the NIST laser at 253.7 nm, the inactivation rates were 0.030 ($p = 1.3 \times 10^{-3}$) for cell culture infectivity and 0.025 ($p = 1.9 \times 10^{-13}$) with LR-qPCR. Using ANCOVA, the adenovirus two dose response measured by DNA damage was not significantly different ($\alpha = 0.05$) from the dose response measured by cell culture infectivity ($p = 0.63$ for LP UV, $p = 0.64$ for NIST laser at 253.7 nm) for both light sources. Additionally, the dose response of adenovirus 2 to LP UV irradiation was not considered statistically different from its response to the NIST laser at 253.7 nm when measured by both infectivity ($p = 0.052$) and LR-qPCR ($p = 0.18$) as determined by ANCOVA. This work verified the equivalency of UV exposure between both UV emission systems.

Four-log inactivation of adenovirus 2 was observed using a LP UV lamp at a UV dose of 174 mJ/cm^2. This is comparable to previous studies, which noted 4-log inactivation at doses between 120 mJ/cm^2 and 168 mJ/cm^2.[3−6] Two-log reduction in DNA amplification of adenovirus 2 was observed using a LP UV dose of 68 mJ/cm^2 when analyzing a fragment size of 1.1 kbp. This differed from two previous studies, which showed 2-log reduction of DNA amplification after approximately 50 mJ/cm^2.[19,20] The difference in dose response could be attributed to inherent variability in adenovirus dose response as reported in the literature, yet could also be due to variations in the methods, such as using different DNA polymerases from different vendors. Previous work on adenovirus DNA damage was performed using rTth polymerase from Applied Biosystems. This present work used GoTaq polymerase and respective buffer from Promega. However, the results in this study demonstrated that the simplified PCR assay, using the hot-start polymerase, could

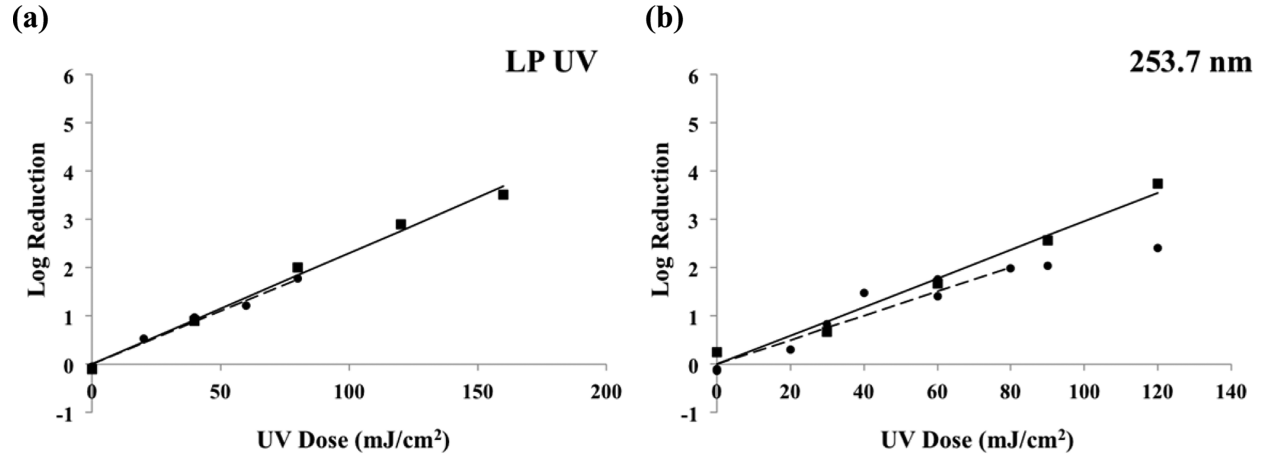

Figure 1. Dose response of adenovirus 2 for irradiation with low-pressure UV light (a) and the NIST laser at 253.7 nm (b) as measured by cell culture infectivity (■) and DNA amplification (●).

achieve a linear relationship of up to 3-log reduction of PCR signal due to UV damage (Figure 1). In addition, the LR-qPCR assay demonstrated good agreement between the reduction in DNA and the reduction in viral infectivity for exposure at 253.7 nm from either source (Figure 1).

Adenovirus is a nonenveloped, icosahedral particle consisting of a protein capsid surrounding a DNA−protein core. Since the viral particle is composed of two primary components: DNA and proteins, loss of infectivity at a given wavelength is assumed to be due to damage to DNA, damage to viral proteins, or both. Inactivation of adenovirus 2 by the NIST laser at 210 nm, 220 nm, 230 nm, 240 nm, 260 nm, 270 nm, 280 nm, and 290 nm is shown in Figure 2 as a function of UV dose. It is important to highlight that the fragments used in the long range PCR for these experiments were 1.1 kbp in length. Therefore, the results should be considered as damage observed in a 1.1 kbp fragment of the adenovirus genome. This sensitivity level allows the levels in reduction in PCR signal to be similar to the levels in reduction in viral infectivity when adenovirus is irradiated with LP UV light, which is the point of comparison. The results indicate that at 240 nm and below, the loss of viral infectivity was significantly greater than the observed damage to the viral genome; therefore, damage to the nucleic acids was not the only cause for the loss of adenoviral infectivity at those wavelengths. At 260 nm, the opposite was true. At 270 nm, 280 nm, and 290 nm, as with 253.7 nm, the sensitivity of the genome and the virus were statistically similar.

Linear inactivation rate constants (cm^2/mJ) for each wavelength are given (Table 1) as determined through infectivity and DNA damage. For the calculations of the inactivation rate constants, some points were not used as indicated in Figure 2. In the case of the infectivity assay, the assay detection limit was reached after obtaining a 5-log reduction; therefore, these points were not used for the calculation of rate. In the case of genome damage determined by LR-qPCR, it was observed previously that tailing occurs after obtaining 2.5- to 3-log reduction in amplifiable genome fragments.[20] This tailing occurs when the genome fragment is saturated with damage and exposed to multiple hits. Multiple damage sites in the same fragment are measured as one point of damage because the PCR can only detect the reduction in amplifiable genome fragments regardless of the number of damage sites per genome. As a result, the log reduction cannot increase and tailing occurs. Therefore, these points were not used for the calculation of rate.

Analysis of covariance (ANCOVA) at each wavelength confirmed a statistical difference ($p < 0.05$) between DNA damage and infectivity at 210 nm, 220 nm, 230 nm, 240 nm, and 260 nm. At 240 nm and below, loss of viral infectivity was significantly greater than the loss of DNA amplification. This implies that damage to a viral component other than the viral genome contributed to loss of infectivity at those wavelengths. The significantly higher loss of infectivity at these low wavelengths is likely due to damage to the viral proteins, which play an integral role in the infection process by initiating adenoviral infection in a suitable host cell, lysing endosomes, and facilitating the release of DNA in the host cell nucleus.[32] At 240 nm and below, the UV absorption of proteins is higher, primarily because of the absorption of peptide bonds, which are prevalent in protein structures as the link between amino acids.[33,34]

At 260 nm, loss of DNA amplification was statistically greater than the loss of viral infectivity. This relatively greater DNA damage could be the result of damaged DNA being repaired during the infectivity assay. Although this phenomenon was not demonstrated directly in this study, the possible role of viral DNA repair has been demonstrated using cell lines with limited repair capability.[13] Repair of photodimeric lesions likely occurred across the germicidal UV spectrum. However, it was not specifically measured in this study in part because the LR-qPCR assay used to measure DNA damage before repair was calibrated to cell culture infectivity, which can be influenced by host cell repair mechanisms. At 254 nm, 270 nm, 280 nm, and 290 nm, differences between loss of viral infectivity and DNA damage were not statistically different, indicating that at those wavelengths loss of viral infectivity is linked primarily to genomic damage. At 280 nm, the UV absorption of proteins has a relative peak due to the absorption of tryptophan and tyrosine amino acids; however, their absorption at 280 nm is an order of magnitude lower than DNA absorption.[34] These results are consistent with the high UV absorption of DNA relative to proteins at those wavelengths.

Figure 3 shows the spectral sensitivity (action spectra) of adenovirus 2 inactivation and its genome damage, obtained by taking the inactivation rate constants relative to their values at 253.7 nm. The figure highlights the significant difference between the rate constants of inactivation and genome damage at wavelengths below 240 nm as well as their relative similarities at wavelengths above 240 nm. At 210 nm, the loss of viral infectivity

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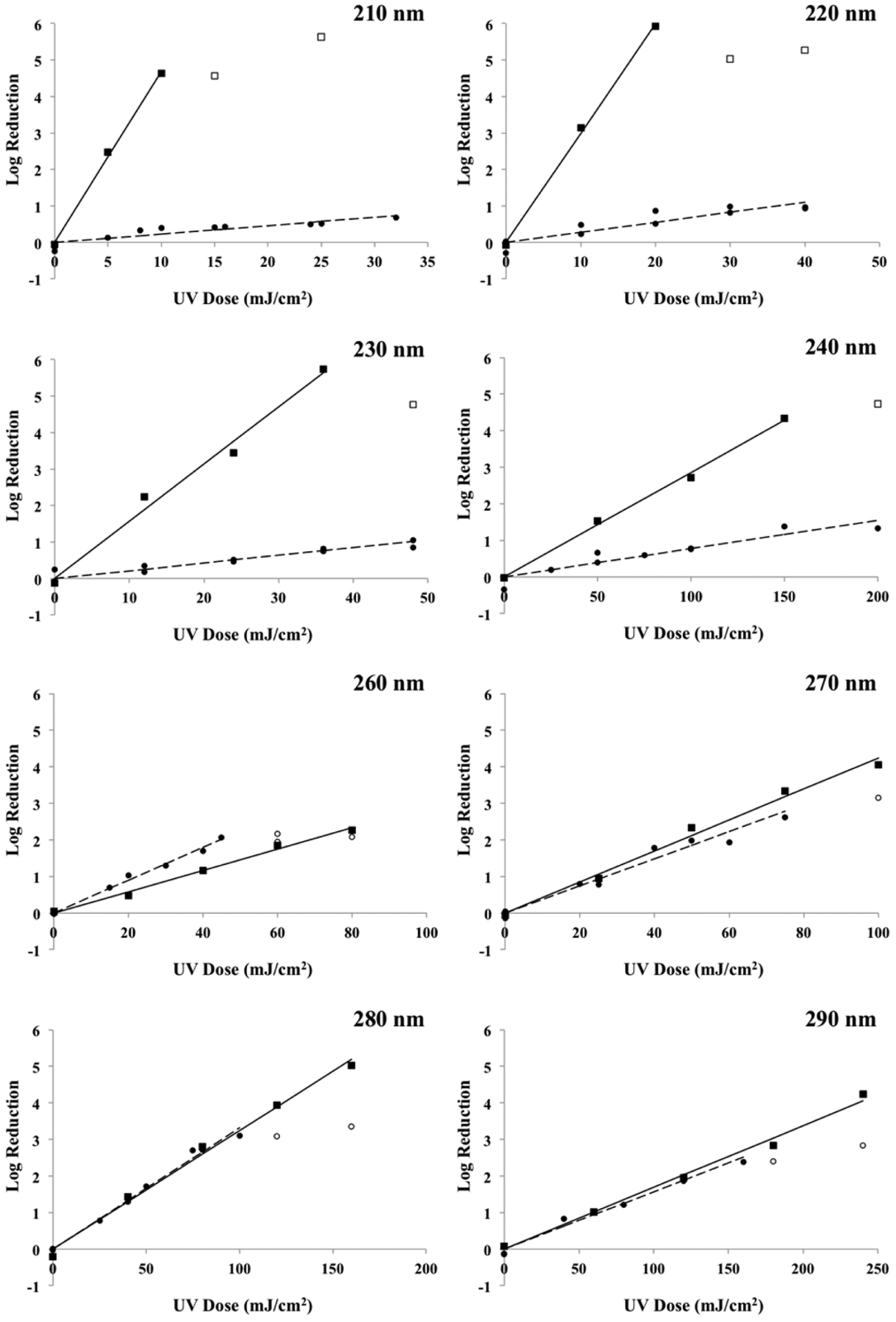

Figure 2. Dose response of adenovirus 2 to monochromatic UV light from the NIST laser at various wavelengths as measured by infectivity (■) and DNA amplification (●). Note different x and y axes labels. Open symbols represent data points not included in statistical analysis due to indications of tailing (genome damage) or from reaching the detection limit (cell culture assay).

was almost 16 times greater than at 254 nm; at 220 nm, it was over 10 times greater. Above 240 nm, the action spectra of inactivation peaked at 270 nm and was relatively flat from 240 to 260 nm. The action spectrum of inactivation follows the same trend as reported in the literature (Figure 3a), which showed five to six times greater inactivation at 222 nm and four to six times greater inactivation at 228 nm.[11] In contrast to this study, the reported action spectrum[11] above 240 nm peaked at 260 nm.

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Table 1. Wavelength-Specific Inactivation Rate Constants (in cm^2/mJ) for Adenovirus 2.

wavelength (nm)	cell culture infectivity: k (±95%CI; p-value)	DNA damage: k (±95%CI; p-value)	ANCOVA[a] p-value
210	0.469 (0.2740; 2.9×10^{-02})	0.023 (0.0108; 1.3×10^{-04})	2.6×10^{-09}
220	0.299 (0.1594; 2.7×10^{-02})	0.028 (0.0110; 8.1×10^{-06})	1.3×10^{-08}
230	0.157 (0.0577; 7.2×10^{-03})	0.021 (0.0054; 2.0×10^{-08})	7.2×10^{-09}
240	0.029 (0.0048; 1.5×10^{-03})	0.008 (0.0016; 6.7×10^{-10})	2.9×10^{-06}
253.7	0.030 (0.0080; 1.3×10^{-03})	0.025 (0.0034; 1.9×10^{-13})	0.64
260	0.029 (0.0045; 2.6×10^{-04})	0.045 (0.0037; 1.1×10^{-12})	1.4×10^{-04}
270	0.042 (0.0081; 4.7×10^{-04})	0.036 (0.0043; 8.7×10^{-15})	0.12
280	0.032 (0.0052; 2.8×10^{-04})	0.033 (0.0020; 7.0×10^{-06})	0.68
290	0.017 (0.0029; 3.5×10^{-04})	0.016 (0.0019; 4.6×10^{-10})	0.43
LP UV	0.023 (0.0043; 4.4×10^{-04})	0.022 (0.0022; 3.3×10^{-09})	0.63

[a]For ANCOVA, significance was defined as $p < 0.05$.

Differences in spectral sensitivity could be attributed to inherent variability in adenovirus dose−response in cell culture as evidenced by the literature; however, they could also be attributed to the UV light sources used. The previous study used MP UV light with bandpass filters, which provided half-peak bandwidths of approximately 10 nm wide compared to less than 1 nm wide for this study.

Figure 3b compares the action spectra of UV inactivation and genome damage to the UV absorption of adenoviral DNA measured in this study. The action spectrum of genome damage and the UV absorption both peaked at 260 nm. This observation is expected because UV energy must be absorbed to cause damage to the genome. At 260 nm, the genome was 1.8 times as sensitive to damage as at 253.7 nm. Above 260 nm, the DNA damage sensitivity decreased with increasing wavelength, which is consistent with a past study of cyclobutane dimer formation at wavelengths between 261 and 305 nm.[15] The adenoviral genome was least sensitive to damage at 240 nm, near the relative minimum of DNA absorption at 230 nm. The viral genome exhibited a higher sensitivity to damage at 220 nm than at 210 nm, which was unexpected. The UV absorption of the intact virus peaks between 210 and 220 nm.[13] It is possible that proteins absorbing at those wavelengths could transfer the UV energy to the DNA. Protein-associated DNA is more susceptible to UV-induced photoproducts than isolated DNA.[35]

The observation that the action spectrum of adenovirus 2 inactivation was relatively flat from 240 to 260 nm but peaked at 270 nm is unusual. Action spectra of other viruses such as rotavirus (dsRNA), MS2 coliphage (ssRNA), T7 coliphage (dsDNA), T1UV Coliphage (dsDNA), and Q beta Coliphage (ssRNA) all show a local minimum at 240 nm and local maximum between 259 and 265 nm.[23,36−39] Other organisms such as *E. coli*, *S. typhimurium*, *P. aeruginosa*, *B. subtilis* also have relative peak sensitivities in the 260−265 nm region.[40−42] The nucleotide base pairs of adenine, thymine, cytosine, and uracil have relative peak absorption values between 260 and 265 nm with guanine peaking closer to 245 and 275 nm.[43] Few action spectra are available for dsDNA viruses. Herpes simplex virus exhibited relative peak sensitivities at 270 and 280 nm;[44] however, herpes simplex has an envelope, which could impact its UV sensitivity. T7 and T1UV, both dsDNA coliphage, showed a peak sensitivity between 260 and 265 nm.[39] However, T7 and T1UV do not infect human cells and their bacterial assays are performed in the dark; dark repair of DNA does not usually occur when the host is growing at log phase.

The lack of increased sensitivity of adenovirus near 260 nm could be an artifact of experimental variability or an indication of DNA damage repair by the host cells used in the infectivity assay. However, statistical analysis demonstrated that adenovirus dose response to 260 nm was statistically different than at 270 nm, but not different than the dose response at 240 or 254 nm; this suggests that the spectral sensitivity is not an artifact of experimental variability. As a double stranded (ds) DNA virus, adenovirus is susceptible to host cell DNA repair, which could affect its infectivity-based spectral sensitivity. At 260 nm, the virus is most sensitive to DNA damage and DNA damage is the dominant mechanism for loss of infectivity; therefore, DNA repair could restore its viral infectivity and affect its infectivity action spectrum. The impact of potential repair of DNA damage in the estimation of UV inactivation of dsDNA viruses during infectivity assays has been investigated with cells deficient in DNA repair mechanisms, demonstrating a significant reduction

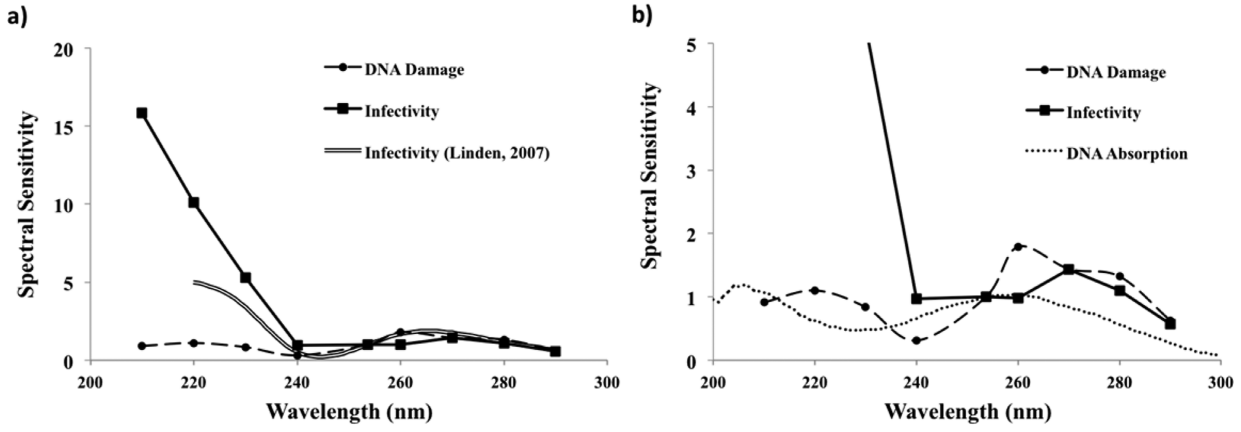

Figure 3. Spectral sensitivity of adenovirus 2 and its nucleic acid (DNA damage) between 210 and 290 nm as compared to (a) the action spectrum in the literature[11] and (b) the UV absorption of adenoviral DNA measured. Note different y-axes values.

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in dose required compared to repair proficient cells.[13] In addition, the adenovirus genome has been demonstrated to sustain damage from LP lamps (254 nm) that did not translate to reduction in viral infectivity,[20] suggesting the potential role of DNA repair mechanisms in the resistance of adenovirus to UV disinfection. The type of DNA damage and potential repair obtained at different wavelengths is not well understood and merits further research.

The action spectrum of adenovirus 2, determined by cell culture infectivity, was used to calculate UV dose delivered by a collimated beam apparatus equipped with a medium pressure lamp. Figure 4 shows the dose response of adenovirus 2 to LP UV and MP UV light. When the MP UV dose was calculated by weighting the MP UV emission spectrum by adenoviral DNA absorption, the LP UV and MP UV results differed considerably (ANCOVA $p = 1.5 \times 10^{-4}$) and MP UV was significantly more effective than LP UV. However, when the MP UV dose was determined by weighting the MP UV emission by the adenovirus action spectrum, the dose response of adenovirus to MP UV light was similar to its response to LP UV light (ANCOVA $p = 0.592$). This theoretical comparison confirms that the adenovirus action spectrum, which was developed relative to 254 nm, is accurate. It also highlights the importance of the low wavelengths for adenovirus disinfection, which are not accounted for as strongly in dosimetry using DNA absorption weighting.

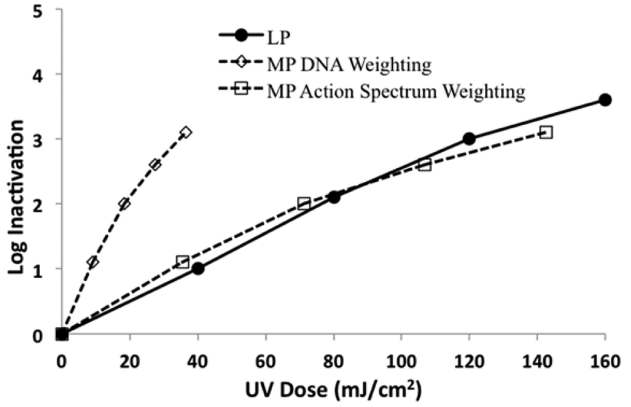

Figure 4. Dose response of adenovirus 2 to LP UV light and MP UV light with the MP UV dose determined by weighting the lamp emission with DNA absorption or the adenovirus action spectrum as measured by cell culture infectivity.

This research provides fundamental insight into the action of ultraviolet light on adenoviruses and the molecular level viral responses to UV irradiation. The data clearly indicate that nucleic acid damage is not the only mechanism responsible for virus inactivation from polychromatic UV lamps, including MP UV lamps, which are commonly used in the water and wastewater treatment industries. It has significant broader impacts with the potential to improve current UV disinfection and system validation, by demonstrating the importance of low wavelengths in inactivating adenoviruses, which drive the UV disinfection requirements. It also has the potential to improve the design of UV technologies by making the case for tailored wavelength units, which could combine a wavelength in the germicidal range, such as 254 or 260 nm, with a wavelength in the protein absorbance region, such as 220 or 230 nm, to optimize pathogen inactivation and minimize energy costs. Future research would complement this study by evaluating the spectral sensitivity of adenoviral proteins, specifically the fiber proteins and DNA binding proteins.

■ ASSOCIATED CONTENT

Supporting Information

In the Supporting Information, Figure S1 shows the emission spectra of the NIST tunable laser at each wavelength used. The cell and virus propagation and adenovirus enumeration methods are described in detail as well as the Long Range and Quantitative Polymerase Chain Reaction steps used to assess genome damage. Table S1 lists the primers and probes used for the LR-qPCR analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(K.G.L.) Phone: 303-492-4798; fax: 303-492-7317; e-mail: karl.linden@colorado.edu.

Notes

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