Rapid inactivation of RNaseA by high irradiance UV LEDs

Theresa Thompson PhD, Jay Paquantonio, Garth Eliason
Phoseon Technology · Hillsboro OR

Abstract

While it has long been known that UV can inactivate enzymes (1), inactivation of RNase is an ongoing challenge for sequencing and other studies reliant upon RNA. A further challenge is the timely inactivation of RNase on laboratory equipment and surfaces. The majority of inactivation strategies require significant investment of time that could be better spent gathering data. UVLED sources can deliver higher directed radiant power than standard mercury lamps. In this study, we constructed an adjustable high intensity 275nm UVLED source and used it to expose RNaseA contaminated dry surfaces (1 µl 0.02 U/ml RNaseA) from a distance of 25 mm. The RNaseA sample was recovered from each surface in RNase free water and the suspension fluorometrically assayed for RNase activity (RNaseAlert IDT). Samples were exposed to 275 nm UVLED for various times and at different irradiances. RNaseA was totally inactivated (as compared to a negative control) with a 3 minute exposure to an irradiance of 38.5 mW/cm² at the exposed surface for a total dose of 6.94 J/cm². Unexpectedly the same total dose at a lower irradiance 18.74 mW/cm² did not inactivate the enzyme to the same extent. Instead a total dose of 22.49 J/cm² delivered over 20 minutes was required to reach negative control levels of enzymatic activity. Further, when the irradiance of the UV LED source was varied from X mW/cm² to Y mW/cm² for a set 5 min exposure time at a fixed distance from the source, the relationship between decreasing enzyme activity and increasing irradiance was nonlinear. This is consistent with numerous studies of bio-inactivation of enzymes and microorganisms (2) that are logarithmic. We conclude that high irradiance UVLED sources can provide faster, more complete control of enzymatic activity in such a way that is impractical with lower-intensity UV sources.

Introduction

In activation of RNase is an ongoing challenge for sequencing and other studies reliant upon RNA. While it has long been known that UV can inactivate enzymes (1), RNaseA is particularly difficult to inactivate, with a significant fraction active after autoclaving, necessitating the use of DEPC treatment or other chemical treatments. Maintenance of RNase-free work areas and surfaces is an ongoing, time consuming challenge and a fast, efficient means of RNase inactivation would be a benefit. UVLED sources can deliver significantly higher directed radiant power than standard mercury UV lamp allowing delivery of higher doses (J/cm²) in shorter time frames.

In this study we investigate the inactivation of RNaseA on a surface using a series of irradiance levels provided by an adjustable high intensity 275 nm UVLED source constructed for this study.

Methods

Lamp Construction:
The variable power, 200 mA, 275 nm UV LED lamp used in these experiments is air-cooled (Phoseon). Lamp power levels were adjustable from 0% through 100% as measured (and monitored) by voltage. The lamp was constructed with continuously adjustable power control allowing control of the dose delivered at target in specific time frames. For this investigation the lamp was positioned either 10 mm or 25 mm from the target surface. Irradiance at the target surface for each power level was measured using a calibrated UV-C power meter and puck.

Slide Preparation
RNaseA (SigmaAldrich) was suspended to a concentration of 0.02 U/ml and 1 µl was spotted onto clean RNase-free glass slides. After UV exposure, RNaseA was recovered in 30 ul RNase-free water followed by a further 30 ul rinse and combined with the intial wash. Recovered samples were assayed for RNase activity using RNaseAlert Kit (IDT) in duplicate on a Gemini XS fluorometer (Molecular Devices).

UV LED exposure
Once completely dry, the contaminated slides were exposed to 275 nm UVLED. UVLED exposure was carried out at 18.74 mW/cm² and at 38.5 mW/cm² (at the target surface) for various times and total dosages. Further series of 5 minute exposures were conducted for 10% 25%, 50%, 75% and 100% power levels. Lamp power was controlled by varying the source voltage and then measured at the emitting window. Dosages were calculated from irradiance measured at the exposed surface and exposure time.

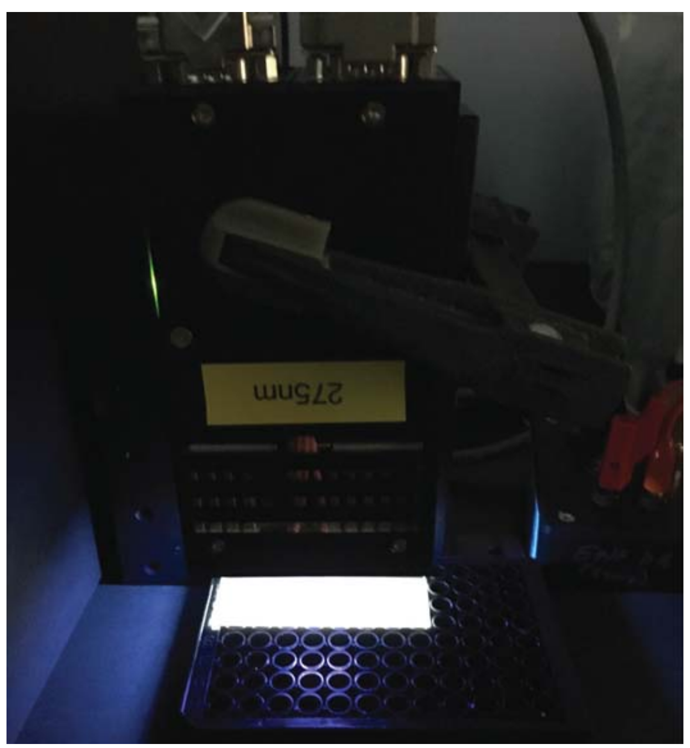

Figure 1
The air-cooled 275 nm UV LED lamp shown here (as used in these experiments) was constructed with continuously adjustable power control allowing control of the dose delivered at target in specific time frames. The lamp has an emitting window size of 75x20mm and, in this photograph, was positioned 10 mm from the glass slide target surface.

Results

In general inactivation of enzymes and microorganisms has been seen as dose dependent phenomenon where Dose=Irradiance X Time and the units are given as Joules/cm² (or Joules/m²). Irradiance (W/cm²) has been treated as a set quantity when investigating UV inactivation of enzymes or log reduction of microorganisms. In these investigations RNaseA was totally inactivated (as compared to a negative control) at a distance of 10 mm with a 3 minute exposure to an irradiance of 38.5 mW/cm² at the exposed surface, for a total dose of 6.94 J/cm². Unexpectedly the same total dose at a lower irradiance 18.74 mW/cm² did not inactivate the enzyme to the same extent. To further investigate this disparity, the lamp power (irradiance) was varied for target exposure time of 5 minutes (Figure 2). As expected, inactivation increased as irradiance increased. We then compared timed inactivation series for two irradiances (50%, 316.7 mW/cm² and 100%, 635.3 mW/cm² at the lamp window). Again, increased exposure time resulted in increased inactivation. However, when RNaseA activity was plotted against total dose delivered for the two irradiances (Figure 3B), higher irradiance effectively inactivated the enzyme at a lower dose, 4.4 J/cm², than lower irradiance which required 25 J/cm². A further plot of percent enzyme activity versus percent lamp power (Figure 4) indicated that the relationship is non-linear, consistent with numerous studies of bio-inactivation of enzymes and microorganisms where the relationship is logarithmic (2).

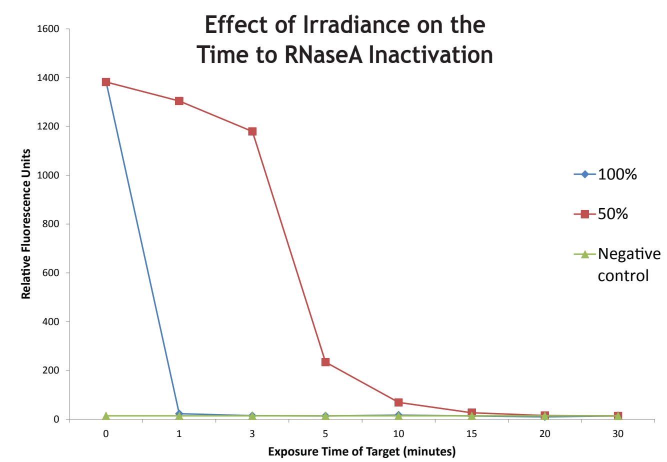

Figure 2A
Effect of Irradiance on the Time to RNaseA Inactivation
RNaseA targets were exposed at 25 mm from the UVLED source at each of two irradiances - 316.7 mW/cm² (50%) and 635.3 mW/cm² (100%).

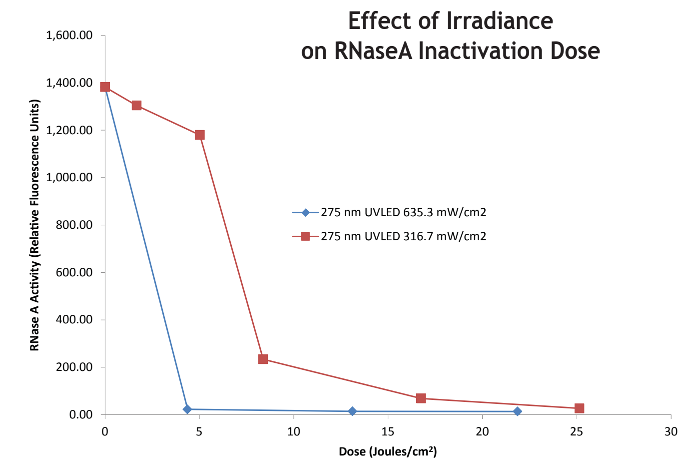

Figure 2B
Effect of Irradiance on RNaseA Inactivation Dose
RNaseA targets were exposed for 0 min (control), 1 min, 3 min, 5 min, 10 min, 15 min, 20 min, and 30 min. Remaining RNaseA activity is reported as relative fluorescence units (IDT RNaseAlert) after a 60 min reaction time at 37°C.

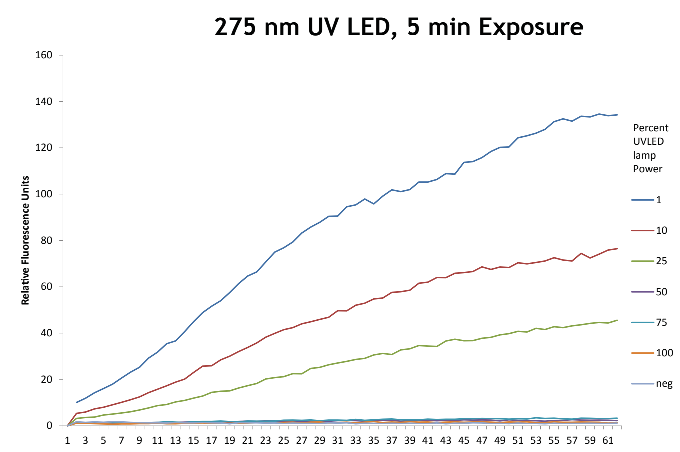

Figure 3
Increased inactivation with increased lamp power. RNaseA contaminated glass slides were exposed to 1%, 10%, 25%, 50%, 75%, and 100% irradiance from a distance of 10 mm. This corresponds to irradiance at the lamp window of 26.4 mW/cm², 50.1 mW/cm², 150.1 mW/cm², 316.7 mW/cm², 477.6 mW/cm² and 635.3 mW/cm².

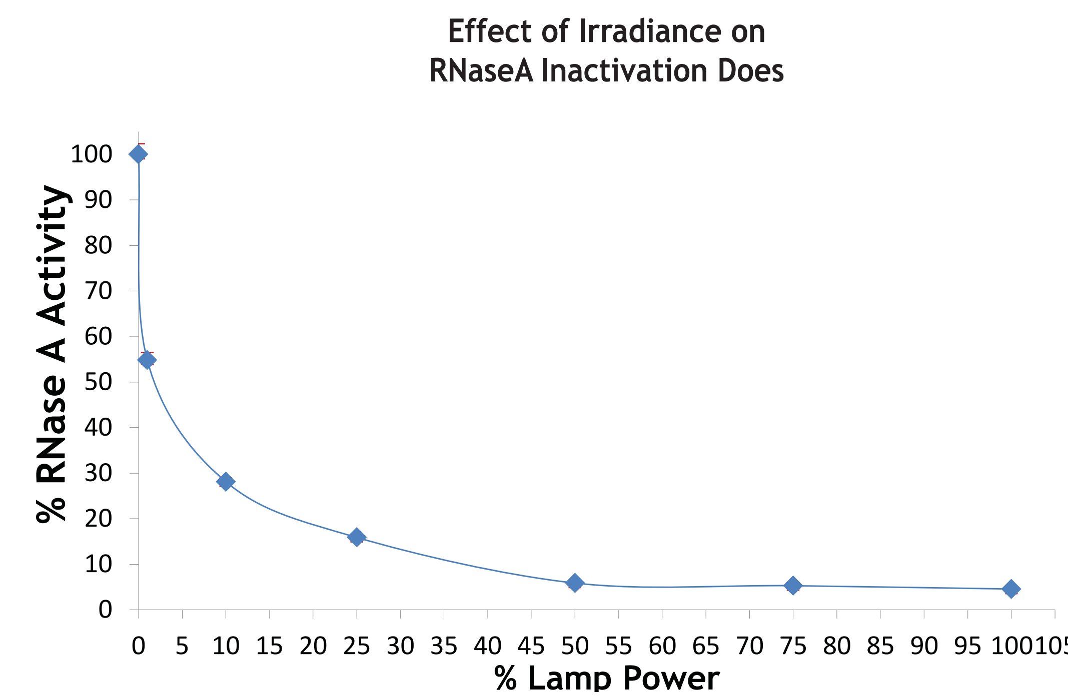

Figure 4
Glass slides contaminated with RNaseA were exposed to 275 nm UVLED for five minutes at seven power levels. Samples were read in a fluorometer using RNaseAlert kit (IDT) to assess initial inactivation response prior to incubation for extended enzyme activity. The initial RNaseA activity was normalized to the activity at 0% lamp power as 100% activity. Source irradiance was measured at the emitting window: 1% (26.4 mW/cm²), 10% (50.1 mW/cm²), 25% (150.1 mW/cm²), 50% (316.7 mW/cm²), 75% (477.6 mW/cm²) and 100% (635.3 mW/cm²). Background levels of RNaseA activity for the negative control (unexposed target) corresponds to 7.3%±0.14%. Percent RNaseA acitivity reaches negative control levels at 50% irradiance.

Conclusions

We conclude that high irradiance UV LED sources can provide faster, more complete control of enzyme activity in a manner impractical for lower-irradiance UV sources.

References

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Chevrefils, G.; Caron, É.; Wright, H.; Sakamoto, G.; Payment, P.; Barbeau, B.; and Cairns, B. 2006. IUVA News, 8(1): 38-45.

Theresa Thompson, PhD
theresa.thompson@phoseon.com
+1 971.246.5766

Phoseon TECHNOLOGY
www.phoseon.com/lifesciences