Irreversible inactivation of RNase A on a surface by UV LED

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Introduction

RNases are pervasive and when contaminating critical surfaces can present problems for laboratories engaged in RNA sequencing and analysis. RNases, specifically RNase A, are difficult to irreversibly inactivate in the absence of long-term heat treatment (1) or harsh chemicals. Such methods may be incompatible with common laboratory materials or complicate subsequent biochemical reactions. UV light at 254 nm is known to act on RNase A via the effect of the aromatic amino acids proximal to disulfide bonds (2). While UV methods for inactivation of RNase A are well known for mercury arc lamp sources, fast, complete, irreversible inactivation has been difficult to achieve.

We report here the first use of high irradiance UV LED sources for enzyme inactivation.

Results

Measured activity of the RNase A decreased to near negative control levels at a dose of 474 J/cm2 from the 316 mW/cm2 275 nm UV LED source (Figure 1). However, the time required to reach this dose (25 min) may be inconveniently long for surface inactivation of RNase in a working laboratory or instrument surface.

RNase A enzyme activity at different timed exposures to 316 mW/cm2 and a higher irradiance 632 mW/cm2 UV LED source is shown in Figure 2. At each time point the UV dose for the 316 mW/cm2 source is one-half that of the 632 mW/cm2 source. Enzyme inactivation is reached at shorter exposure times with the higher doses possible using higher irradiance. Further, equivalent doses from the two sources did not inactivate RNase A to the same extent. For example a direct comparison of the 5 min 632 mW/cm2 and 10 min 316 mW/cm2 time points suggests that, at equivalent doses (190 J/cm2), the higher irradiance source is more effective at inactivating the enzyme.

Exposure to 316 mW/cm2 275 nm source decreased Vmax from 163 RFU/min at a 3 minute exposure and 9 RFU/min for a 15 minute exposure to reach RNase A negative control levels at a 30 minute exposure. Illumination with the 635.4 mW/cm2 275 nm source reduced Vmax to match the RNase A negative control at an exposure time of less than 5 minutes.

Total radiant exposure to reach RNase A activity equivalent to the negative controls was 114 J/cm2 for the 635.4 mW/cm2 source and 474 J/cm2 for the 316 mW/cm2 source (Figure 3).

In a separate experiment (not shown) samples inactivated to match negative control (189 J/cm2 with the 632 mW/cm2 UVLED source) were held for 24 hours in a dark box before being resuspended; assayed activity remained at negative control levels. No reactivation of RNase A was noted.

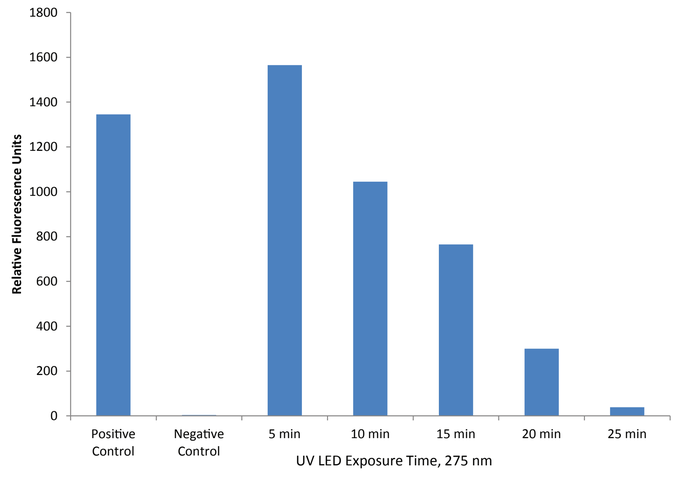


Figure 1
Inactivation of RNase A by increasing UV dose from a 275 nm 316 mW/cm2 UV LED source. RNase A dried onto glass slides was exposed to UV LED at 316 mW/cm2, for doses of 56.8 (3 min), 189.6 (10 min), 284.4 (15 min), 379.2 (20 min), and 474 J/cm2 (25 min). For the Negative Control, RNase-free water was recovered from an untreated clean slide. For the Positive Control, RNase A was applied to a clean slide and allowed to dry and subsequently recovered. Data shown are representative.

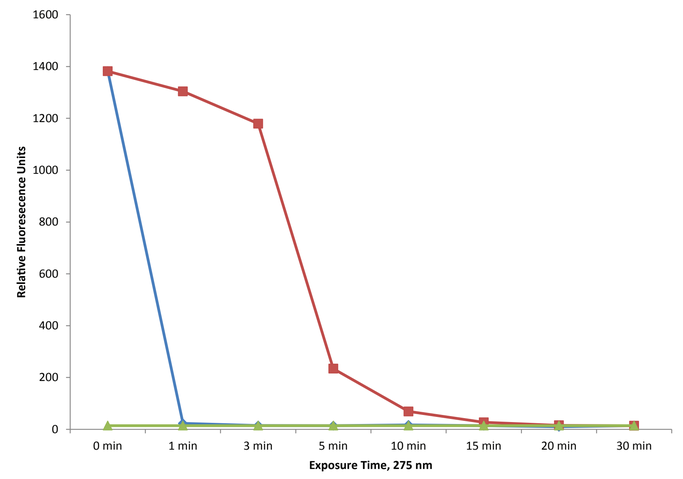
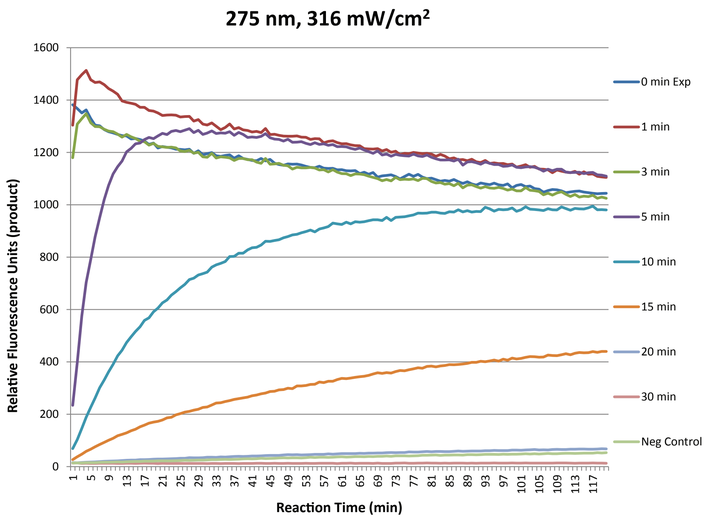


Figure 2
Comparison of time to inactivate RNase A at two irradiances. Negative control – no RNase A (green triangles), Low irradiance 275 nm source - 316 mW/cm2 (red squares) and, high irradiance source - 635.4 mW/cm2 (blue diamonds). Reactions were incubated for 30 min prior to measuring fluorescence.



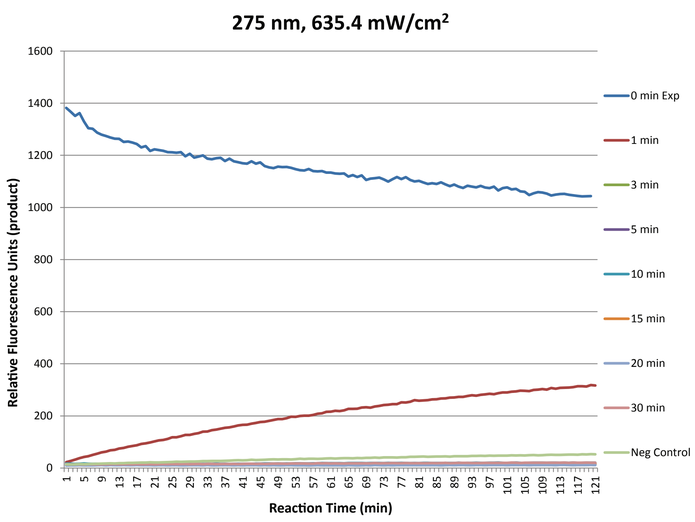


Figure 3
Inactivation Curves

A: 275 nm 316 mW/cm2. Exposure times correspond to the following doses: 0 min(0 J/cm2), 1 min (19 J/cm2), 3 min (57 J/cm2), 5 min (95 J/cm2), 10 min (190 J/cm2), 15 min (284 J/cm2), 20 min (379 J/cm2), 25 min (474 J/cm2), 30 min (569 J/cm2).

B: 275 nm 632 mW/cm2. Exposure times correspond to the following doses: 0 min (0 J/cm2), 1 min (38 J/cm2), 3 min (114 J/cm2), 5 min (190 J/cm2), 10 min (379 J/cm2), 15 min (569 J/cm2), 20 min (758 J/cm2), 25 min (948 J/cm2), 30 min (1138 J/cm2).

Materials & Methods

RNase A (Worthington) was suspended at 0.02 U/ml (2.47µg/ml) in sterile RNase-free water immediately prior to use. Clean glass slides were spotted with 2 µl RNase A solution and allowed to dry.

Dried sample slides were exposed to a 275 nm UV LED source (Phoseon Tehnology) at 25 mm from the source. Lamp irradiance was set at either 316 mW/cm2 or 635.4 mW/cm2. Exposure times ranged from 0 to 30 minutes. Samples were held in a dark box until resuspended for assay at the conclusion of each set of exposures.

Treated Rnase A was resuspended from slides in 2 X 30 µl RNase-free water and transferred to microtiter plates.

RNase A activity of the resuspended samples was assayed using the RNaseAlert substrate and buffer (IDT). Fluorescence was detected on a GeminiXS microplate fluorometer (Molecular Devices). Reactions were monitored for either one or two hours at one minute intervals, or as described in Figure legends.

Literature Cited

Zale and Klibanov, Why does ribonuclease irreversibly inactivate at high temperatures. Biochem 25:5432-5444 (1986).

T.K. Rathinasamy and L.G. Augenstein, Photochemical yields in ribonuclease and oxidized glutathione irradiated at different wavelengths in the ultraviolet. Biophys. J. 8:1275-1287 (1968).

Conclusions

These results show that both radiant flux (power) and radiant fluence (dose) contribute to rapid inactivation of the RNase A enzyme. We conclude that high power 275 nm UV LED irradiation represents a novel, fast and convenient irreversible inactivation method for RNases on surfaces.

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