Synergy Between 275 nm and 365 nm UV LEDs for Inactivation of RNase A

Theresa Thompson PhD, Garth Eliason & Jay Pasquantonio

Phoseon Technology · Hillsboro OR

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Overview:

- Investigate the interaction of 275 nm and 365 nm high irradiance UV LED sources on enzyme inactivation
- Wavelengths chosen are targeted to disrupt the disulfides (275 nm) and lysine amino acid side chains (365 nm).
- RNase A, a highly stable and denaturation resistant enzyme, was the target for inactivation
- Dried samples of the target were exposed to high irradiance UV LED sources
- Fluorescent assays of RNase A showed decreased enzyme activity on exposure to UV LED sources
- The effects of the 275 nm and 365 nm UV LED sources were found to be synergistic

Introduction:

We have recently determined that a high irradiance 275 nm UV LED source inactivates RNaseA at lower doses (joules/cm2) than lower irradiance source (1). Inactivation using 275 nm disrupts the disulfide bonds (2) but does not directly affect the histidine or lysine amino acids known to be located in the enzyme's active site. In this study, 365 nm UV LED (a wavelength that approximates the bond dissociation energy of 331 kJ/mol for CH3-NH2 group present in the lysine amino acid side chain) was investigated for its effect on RNaseA enzyme activity.

Methods:

RNaseA contaminated dry surfaces (1 µl 0.02 U/ml RNaseA) were exposed to UV LEDs 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). RNaseA samples were exposed to 275 nm alone (high irradiance - 38.5 mW/cm2 and low irradiance - 18.7 mW/cm2 at the target), to 365 nm alone (1.35 W/cm2 at the target), and to 275 nm and 365 nm concurrently (18.7 mW/cm2 and 1.35 W/cm2, respectively).

Results:

The 275 nm UV LEDs inactivated RNaseA at doses of 6.9 J/cm2 for the 38.5 mW/cm2 275 nm source and 22.5 J/cm2 for the 18.7 mW/cm2 275 nm source. Exposure to 365 nm alone at doses up to 2400 J/cm2 had no effect on enzyme activity - tracking the positive control (Fig 3) When concurrently exposed to the low irradiance 275nm and the 365 nm sources, RNaseA was inactivated at a dose of 5.6 J/cm2 (275 nm source) and 406 J/cm2 (365 nm source).

Conclusions

This result shows a synergy between the effects of 275 nm and 365 nm wavelengths for RNaseA inactivation, suggesting that the use of multiple targeted wavelengths is a viable path for control and rapid inactivation of proteins and enzymes.

Literature Cited

1) Pasquantonio J., Eliason G., and Thompson T. Rapid Inactivation of RNase A by High Irradiance UV LEDs (Submitted).
2) Neves-Peterson MT, Gryczynski Z, Lakowicz J, Fojan P, Pederson S, and Bjørn. 2002. High probability of disturbing a disulfide bridge mediated by endogenous excited tryptophan residue, Protein Sci. 11(3): 588-600.

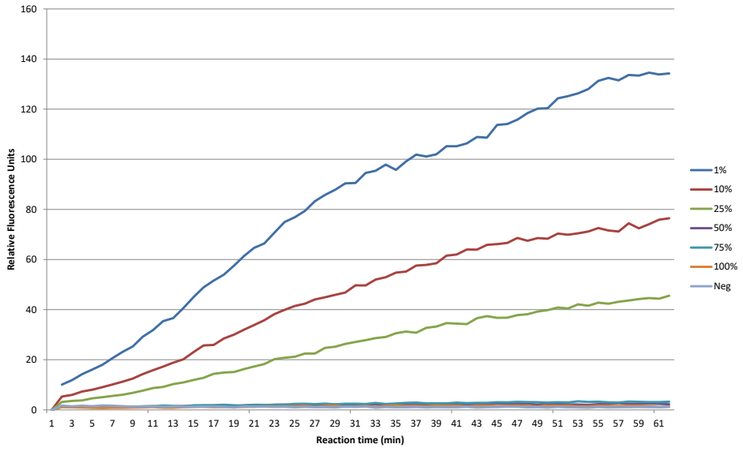

Figure 1

275 nm UV LED, 5 min Exposure, Varied Irradiance Levels

275 nm UV LED, 5 min Exposure, Varied Irradiance Levels. Dried samples of RNase A were exposed to 275 nm UV LED for 5 minutes. Irradiance levels were varied for each exposure by varying the source voltage from 1 to 10 volts. Irradiance at the target varied with changes in the source voltage as follows: 1% (est. 12 mW/cm²), 10% (108.3 mW/cm²), 25% (345.7 mW/cm²), 50% (low irradiance - 692 mW/cm²), 75% (1090 mW/cm²), and 100% (high irradiance - 1354 mW/cm²). Irradiance was measured at 25 mm from the 275 nm UV LED source.

	275 nm @ 100% (*38.5 mW/cm²)	275 nm @50% (*18.7 mW/cm²)	365 nm @100% (*1.35 W/cm²)
Seconds	Dose (mJoules)	Dose (mJoules)	Dose (mJoules)
60	2313.84	1124.52	81240
180	6941.52	3373.56	243720
300	11569.20	5622.6	406200
600	23138.40	11245.2	812400
900	34707.60	16867.8	1218600
1200	46276.80	22490.4	1624800
1500	57846.00	28113	2031000
1800	69415.20	33735.6	2437200
2100	80984.40	39358.2	2843400

*Measured at a 45° angle from a distance of 25 mm Measurement at 90° for optimal function of the detector gave values of 205 mW/cm² (50% 275 nm), 316 mW/cm² (100% 275 nm), and 500 mW/cm² (25% 365 nm).

Figure 2

Doses for High Irradiance 275 nm, Low Irradiance 275 nm, and 365 nm UV LEDs

Figure 2 Legend: Doses for High Irradiance 275 nm, Low Irradiance 275 nm, and 365 nm UV LEDs
The mJoule doses where RNase A inactivation occurs. Purple indicates inactivation with 38.5 mW/cm² 275 nm UV LED (6.9 Joules) or 18.7 mW/cm² 275 nm UV LED (5.6 Joules). Green highlights indicate the doses of 18.7 mW/cm² 275 nm UV LED (5.6 Joules) and 1.3 W/cm² 365 nm UV LED (406 Joules) when used simultaneously.

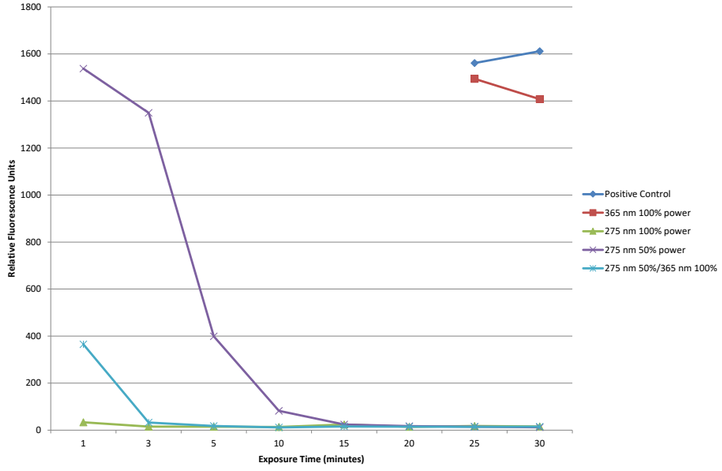

Figure 3

Combined effects of 275 nm and 365 nm UV LED Sources on RNase A time to inactivation

The time to inactivation of RNase A using 275 nm UV LED sources is, predictably, shorter with higher irradiance. However, actual dose required is ~3 fold lower. The combination of 365 nm UV LED and 275 nm UV LED (18.7 mW/cm²) results in a further reduction of the 275 nm dose required for inactivation to 5.6 Joules. This is true even though the dose from the 365 nm is insufficient to appreciably inactivate RNase A (closely tracking the positive enzymatic activity control).

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

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